Vol. XXII. *March, 1912.* *No. 4*

BIOLOGICAL BULLETIN

"STRAINS" IN HYDATINA SENTA.

D. D. WHITNEY.

In a former paper results of experiments upon two races of the rotifer *Hydatina senta* were given in regard to the production of one hundred generations of females without the appearance of males in either race. These experiments have been extended further for about seventeen months and as they are concluded it seems desirable to record the results obtained partly as a confirmation of the earlier conclusions and partly because they form evidence which shows that there exists different races or strains or lines within this particular species of *Hydatina senta*.

In the former paper it was shown how readily male-producing females could be produced in newly made dilute uncooked horse manure cultures and also how readily the male-producing females could be repressed in newly made concentrated cooked horse manure cultures.

In the present paper the parallel history of three races of rotifers *A*, *B*, and *C* is given. Races *B* and *C* are the same races upon which the former conclusions were based while race *A* is an additional one. Races *A* and *B* are sister races, both having developed from one fertilized egg while race *C* is unrelated to races *A* and *B* except in as far as all three races came from the same general culture of rotifers which was originally collected at Grantwood, New Jersey, in 1906.

Races *A* and *B* were always conducted in a parallel series but race *C* was not put into the parallel series until it was in the 36th generation. During this early period of the three races before they were all put into the parallel series the food was from miscellaneous protozoa cultures of various ages made in dilute uncooked horse manure media. The summary of the early history

of these three races before they were all conducted in the parallel series is recorded in Table I. The percentage of male-producing females of races *A* and *B* are practically equivalent, while that of race *C* is much lower.

TABLE I.

Showing the number of female- and male-producers in the three races *A*, *B*, and *C*, from their origin to the time at which parallel records were taken. Female-producers are designated ♀♀, male-producers ♂♀.

Race.	Generations.	No. of ♀♀.	No. of ♂♀.	Per Cent. of ♂♀.	Time.	Food.
A	1–144	1,188	181	13.22+	Oct. 6, 1908, to Aug. 31, 1909.	Dilute uncooked horse manure media, 7–28 days old. Miscellaneous protozoa growing in them.
B	1–146	1,224	167	12.00+	Oct. 6, 1908, to Aug. 31, 1909.	
C	1– 35	210	10	4.54+	June 16, 1909, to Aug. 31, 1909.	

September 3, 1909, these three races *A*, *B*, and *C* were started in a parallel series under as identical external conditions as possible. At the beginning of this parallel series the generations were renumbered and the beginning generation of each of the races in this series is called No. I. Ten young females from each

TABLE II.

Race *C*.

Generation.	No. of ♀♀.	No. of ♂♀.	Per Cent. of ♂♀.	Time.	Food.
1	9	3	25	June 16, 1909, June 18, 1909.	Same as Table I.
2	20	0	0	June 18, 1909, June 20, 1909.	
3	16	4	20	June 21, 1909, June 22, 1909.	
4	8	2	20	June 24, 1909, June 26, 1909.	
Partial summary.	53	9	14.51+		
5–34	150	0	0	June 26, 1909, Aug. 30, 1909.	
35	7	1	12.5	Aug. 30, 1909, Sept. 1, 1909.	
Total summary.	210	10	4.54+		

Detailed history of race *C* throughout the first 35 generations, which is also summarized in Table I.

generation of each race were isolated at the same time and each female placed in a Syracuse watch glass and allowed to mature and to produce daughter females. Then this process was repeated for 345 generations. All the females at each isolation were placed in the same quantity of tap water to which was added the same amount of food culture that was taken from one food jar. The watch glasses in which the rotifers lived always were in three stacks side by side at room temperature. Practically all external influences were as identical as it was possible to make them.

The detailed observations are given in Table III. in parallel columns and the summary is given in Table IV.

At the end of Table I., races *A* and *B*, which up to this time were fed on various protozoa cultures, were practically identical in regard to the percentages of male-producing females in each race, but at the beginning of Tables III. and IV. when the two races were subjected to uncooked concentrated food culture media a decided change occurred. Race *A* retained and even exceeded its former rate of production of male-producing females, but in race *B* the rate was very perceptibly lowered. Race *C* lowered slightly its rate of male-producing females. This occurred during the first 56 generations. From the 57th to the 345th generation in races *A* and *C* and to the end of race *B*, the 239th generation, concentrated cooked food media was used and caused a decided lowering of the production of male-producing females in all races. In race *C* this was reduced to zero, in race *B* to less than 1 per cent., and in race *A* to about 3.5 per cent.

This confirms the earlier results in showing that it is possible by external conditions to repress entirely the production of male-producing females in some races of this rotifer for a long period of time. In race *C* the male-producing females were repressed for 289 generations and then reappeared when the food media was made too dilute accidentally.

If these three races were exactly alike in their power to produce male-producing females and all were subjected to the same external conditions they ought to produce such male-producing females at the same rate. However, as the above observations show that the rates of production of male-laying females vary

TABLE III.

Showing number of female- and male-producers in a parallel series of 345 generations in the three races *A*, *B*, and *C*. Generations 1–56 show the detailed results when the three races were fed upon concentrated uncooked food media and generations 57–345 show the detailed results when the same three races were fed upon concentrated cooked media.

Generation.	Race *A*.		Race *B*.		Race *C*.	
	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.
1	5	0	6	0	7	0
2	8	2	10	0	10	0
3	9	0	10	0	9	0
4	9	0	9	0	9	0
5	9	0	8	0	9	0
6	9	0	9	0	9	0
7	9	0	9	0	9	1
8	10	0	9	0	10	0
9	8	2	10	0	10	0
10	8	2	10	0	9	1
11	9	1	10	0	10	0
12	10	0	10	0	10	0
13	10	0	10	0	10	0
14	7	3	10	0	9	1
15	9	1	9	1	10	0
16	7	3	8	1	10	0
17	10	0	10	0	10	0
18	9	1	10	0	10	0
19	10	0	9	0	9	1
20	9	1	10	0	10	0
21	8	2	10	0	8	2
22	8	2	9	1	10	0
23	9	1	8	1	10	0
24	8	2	10	0	10	0
25	9	1	10	0	10	0
26	9	1	9	1	9	1
27	5	5	10	0	10	0
28	10	0	10	0	9	0
29	10	0	10	0	9	1
30	8	2	10	0	10	0
31	5	5	9	1	10	0
32	10	0	8	2	10	0
33	10	0	10	0	10	0
34	4	6	10	0	10	0
35	6	4	9	0	10	0
36	6	3	10	0	10	0
37	9	1	10	0	10	0
38	8	2	10	0	10	0
39	9	1	10	0	9	1
40	1	9	6	4	6	4
41	7	3	10	0	10	0
42	8	2	10	0	10	0
43	7	3	10	0	10	0
44	7	3	10	0	9	1
45	7	1	9	0	9	0
46	7	3	10	0	10	0
47	7	3	9	1	10	0

TABLE III.—*Continued.*

Generation.	Race *A*.		Race *B*.		Race *C*.	
	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.
48	7	3	10	0	9	1
49	6	3	9	1	10	0
50	7	3	9	1	10	0
51	7	3	10	0	10	0
52	7	2	10	0	10	0
53	10	0	10	0	10	0
54	9	1	10	0	10	0
55	10	0	10	0	9	1
56	6	4	9	1	8	2
57	10	0	10	0	10	0[1]
58	10	0	10	0	10	0
59	8	2	10	0	10	0
60	4	6	10	0	10	0
61	8	2	10	0	10	0
62	4	6	10	0	10	0
63	7	2	10	0	10	0
64	10	0	10	0	10	0
65	10	0	10	0	10	0
66	9	1	10	0	10	0
67	8	2	10	0	10	0
68	10	0	10	0	10	0
69	10	0	10	0	10	0
70	10	0	10	0	10	0
71	5	3	8	0	10	0
72	10	0	10	0	10	0
73	10	0	10	0	10	0
74	10	0	10	0	10	0
75	10	0	10	0	10	0
76	10	0	10	0	10	0
77	10	0	10	0	10	0
78	10	0	10	0	10	0
79	10	0	10	0	10	0
80	10	0	10	0	10	0
81	10	0	10	0	10	0
82	9	1	10	0	10	0
83	10	0	10	0	10	0
84	10	0	10	0	10	0
85	10	0	10	0	10	0
86	10	0	10	0	10	0
87	10	0	10	0	10	0
88	10	0	10	0	10	0
89	10	0	10	0	10	0
90	10	0	10	0	10	0
91	9	1	10	0	10	0
92	10	0	10	0	9	0
93	10	0	10	0	10	0
94	10	0	10	0	10	0
95	10	0	10	0	10	0
96	10	0	10	0	10	0
97	10	0	10	0	10	0
98	10	0	10	0	10	0
99	10	0	10	0	10	0

[1] Concentrated cooked horse manure media began to be used.

TABLE III.—*Continued.*

Generation.	Race *A*.		Race *B*.		Race *C*.	
	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.
100	9	1	10	0	10	0
101	10	0	10	0	10	0
102	10	0	10	0	10	0
103	10	0	10	0	10	0
104	10	0	10	0	10	0
105	9	1	10	0	10	0
106	10	0	10	0	10	0
107	10	0	10	0	10	0
108	10	0	10	0	10	0
109	10	0	10	0	10	0
110	10	0	10	0	10	0
111	10	0	10	0	6	0
112	10	0	10	0	10	0
113	10	0	10	0	10	0
114	10	0	10	0	10	0
115	10	0	10	0	10	0
116	10	0	10	0	10	0
117	10	0	10	0	10	0
118	10	0	10	0	10	0
119	10	0	10	0	10	0
120	10	0	10	0	10	0
121	10	0	10	0	10	0
122	10	0	10	0	10	0
123	10	0	10	0	10	0
124	10	0	10	0	10	0
125	10	0	10	0	10	0
126	10	0	10	0	10	0
127	10	0	10	0	10	0
128	10	0	10	0	10	0
129	10	0	10	0	10	0
130	10	0	10	0	10	0
131	10	0	10	0	10	0
132	10	0	10	0	10	0
133	10	0	10	0	10	0
134	10	0	10	0	10	0
135	10	0	10	0	10	0
136	10	0	10	0	10	0
137	10	0	10	0	10	0
138	10	0	10	0	10	0
139	10	0	10	0	10	0
140	10	0	10	0	10	0
141	10	0	10	0	10	0
142	10	0	10	0	10	0
143	10	0	10	0	10	0
144	10	0	10	0	10	0
145	10	0	10	0	10	0
146	10	0	10	0	10	0
147	10	0	10	0	10	0
148	10	0	10	0	10	0
149	10	0	10	0	10	0
150	10	0	10	0	10	0
151	10	0	10	0	10	0
152	10	0	10	0	10	0
153	10	0	10	0	10	0

TABLE III.—*Continued.*

Generation.	Race *A*.		Race *B*.		Race *C*.	
	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.
154	10	0	10	0	10	0
155	10	0	10	0	10	0
156	10	0	10	0	10	0
157	10	0	10	0	10	0
158	10	0	10	0	10	0
159	10	0	10	0	10	0
160	10	0	10	0	10	0
161	10	0	10	0	10	0
162	10	0	10	0	10	0
163	10	0	10	0	10	0
164	10	0	10	0	10	0
165	10	0	10	0	10	0
166	9	1	10	0	10	0
167	9	1	10	0	10	0
168	10	0	10	0	10	0
169	10	0	10	0	10	0
170	10	0	10	0	10	0
171	10	0	10	0	10	0
172	10	0	10	0	10	0
173	10	0	10	0	10	0
174	10	0	10	0	10	0
175	10	0	10	0	10	0
176	10	0	10	0	10	0
177	10	0	10	0	10	0
178	10	0	10	0	10	0
179	10	0	10	0	10	0
180	10	0	10	0	10	0
181	10	0	10	0	10	0
182	10	0	10	0	10	0
183	10	0	10	0	10	0
184	10	0	10	0	10	0
185	10	0	10	0	10	0
186	10	0	10	0	10	0
187	10	0	10	0	10	0
188	10	0	10	0	10	0
189	10	0	10	0	10	0
190	8	2	10	0	10	0
191	10	0	10	0	10	0
192	10	0	10	0	10	0
193	2	0	2	0	1	0
194	1	0	1	0	1	0
195	4	0	10	0	10	0
196	6	0	8	0	3	0
197	10	0	10	0	10	0
198	10	0	10	0	10	0
199	10	0	10	0	10	0
200	10	0	10	0	10	0
201	10	0	10	0	10	0
202	10	0	10	0	10	0
203	9	1	10	0	10	0
204	9	1	10	0	10	0
205	10	0	10	0	10	0
206	10	0	10	0	10	0
207	10	0	10	0	10	0

TABLE III.—*Continued.*

Generation.	Race *A*.		Race *B*.		Race *C*.	
	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No of ♂♀.
208	10	0	10	0	10	0
209	10	0	10	0	10	0
210	10	0	10	0	10	0
211	10	0	10	0	10	0
212	10	0	9	1	10	0
213	10	0	10	0	10	0
214	10	0	10	0	10	0
215	10	0	10	0	10	0
216	8	0	8	0	8	0
217	9	1	10	0	10	0
218	10	0	10	0	10	0
219	10	0	10	0	10	0
220	10	0	10	0	10	0
221	10	0	10	0	10	0
222	5	0	4	0	10	0
223	10	0	10	0	10	0
224	10	0	10	0	10	0
225	10	0	10	0	10	0
226	10	0	9	1	10	0
227	1	0	1	0	1	0
228	10	0	10	0	10	0
229	10	0	10	0	10	0
230	10	0	4	6	10	0
231	10	0	10	0	10	0
232	10	0	5	0	10	0
233	10	0	10	0	10	0
234	7	0	4	0	9	0
235	1	0	2	0	10	0
236	4	0	1	0	3	0
237	10	0	1	0	10	0
238	10	0	4	0	10	0
239	10	0	0	0	9	0
240	10	0	Died.		10	0
241	3	0			10	0
242	4	0			10	0
243	10	0			10	0
244	8	2			10	0
245	10	0			10	0
246	10	0			10	0
247	10	0			10	0
248	10	0			10	0
249	10	0			10	0
250	10	0			10	0
251	10	0			10	0
252	9	0			10	0
253	10	0			10	0
254	9	0			9	0
255	10	0			10	0
256	6	0			9	0
257	10	0			10	0
258	10	0			10	0
259	10	0			10	0
260	10	0			10	0
261	10	0			10	0

TABLE III.—*Continued.*

Generation.	Race *A*.		Race *B*.		Race *C*.	
	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.
262	7	0			10	0
263	9	0			10	0
264	9	0			10	0
265	10	0			10	0
266	10	0			10	0
267	10	0			10	0
268	10	0			10	0
269	10	0			10	0
270	10	0			10	0
271	10	0			10	0
272	10	0			10	0
273	10	0			10	0
274	7	3			10	0
275	10	0			10	0
276	10	0			10	0
277	10	0			10	0
278	10	0			10	0
279	10	0			10	0
280	8	0			10	0
281	9	1			10	0
282	8	2			10	0
283	8	2			10	0
284	10	0			10	0
285	4	6			10	0
286	5	4			10	0
287	9	1			10	0
288	8	2			10	0
289	6	0			10	0
290	7	0			7	0
291	6	1			9	0
292	8	0			2	0
293	9	0			10	0
294	10	0			10	0
295	4	0			9	0
296	4	0			9	0
297	1	0			9	0
298	4	1			9	0
299	4	5			4	0
300	8	0			8	0
301	7	0			10	0
302	10	0			10	0
303	9	0			8	0
304	10	0			10	0
305	9	0			8	0
306	10	0			8	0
307	8	0			9	0
308	4	0			7	0
309	6	0			10	0
310	7	0			10	0
311	10	0			10	0
312	5	0			5	0
313	5	0			5	0
314	5	0			5	0
315	3	2			5	0

TABLE III.—*Concluded.*

Generation.	Race *A*.		Race *B*.		Race *C*.	
	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.	No. of ♀♀.	No. of ♂♀.
316	4	1			5	0
317	5	0			5	0
318	5	0			5	0
319	10	0			10	0
320	8	2			10	0
321	5	0			5	0
322	5	0			5	0
323	4	1			5	0
324	5	0			5	0
325	5	0			10	0
326	9	1			10	0
327	6	2			10	0
328	3	0			10	0
329	9	1			10	0
330	10	0			10	0
331	7	3			10	0
332	10	0			10	0
333	8	2			10	0
334	6	4			10	0
335	8	0			10	0
336	8	2			10	0
337	8	2			10	0
338	9	1			10	0
339	10	0			10	0
340	10	0			10	0
341	10	0			10	0
342	10	0			10	0
343	4	2			10	0
344	9	0			10	0
345	5	1			10	0
346	6	0			8	2[1]
347	8	0			10	0
348	5	0			9	1
349	3	0			9	1

in the three strains, *A*, *B*, and *C*, when all external conditions are identical the only conclusion that can be drawn is that the three strains differ at least in regard to this single characteristic. Perhaps they all may be potentially alike in their capacity to produce male-laying females but some races may be more easily effected than other races by the influence which causes male-producing females to be produced.

Whenever races *A* and *B* were put into newly made diluted uncooked culture media in battery jars great numbers of fertilized eggs were produced in both races. From general observations they seemed to be produced in equal numbers, thus seeming to

[1] Food media was diluted accidentally.

form evidence that these two races were potentially alike in their power to produce male-producing females but when conditions were unfavorable they differed, as shown in the parallel series, in their responsiveness to the influences that so acted upon the females as to cause them to produce male-producing daughter females. However, when race *C* was put into newly made uncooked culture media in battery jars very few fertilized eggs were produced, thus seeming to show either that this race *C* was potentially different from the other two races in its capacity to produce male-producing females or that it was not as easily acted upon by the male-producing female influences as were races *A* and *B*. Notwithstanding this fact that race *C* produced very few male-producing females when put into battery jars containing dilute uncooked horse manure media it should be stated that in the early history of race *C* it had as high a percentage of male-producing females in the first four generations as was found in either race *A* or *B*, Table II. The race was isolated from a general culture jar in which an abundance of males were appearing at the time of isolation. Beginning with generation five very few males appeared thereafter. This early history shows that race *C* at one time was as potential in its power to produce male-producing females as races *A* and *B*, but whether it later lost this power or never was again subjected to as favorable influences for the production of male-producing females it is impossible to state. Whatever may be the true explanation of this divergence in the male-producing female rates of the three races it surely indicates a difference in the races either in their capacity to produce male-producing females or their responsiveness to the influences that cause male-producing females to be produced.

Punnett concluded that he found "sex strains" in *Hydatina senta* which differed in their power to produce males and even concluded that he found some strains that produced no males. It is very possible that such maleless strains were really like race *C* in the above experiments. From observations and experiments published in an earlier paper ('07), it was shown that no pure female strains could be found. The results of the present experiments corroborate this earlier conclusion. However, the evidence at that time showed no strains of any kind but the

TABLE IV.

Summary of Table III. showing the influence of concentrated uncooked horse manure media containing various protozoa and concentrated cooked horse manure media containing only the protozoa, *Polytoma*.

Generations.	Race *A*.			Race *B*.			Race *C*.			Time.	Food.
	No of ♀♀.	No. of ♂♀.	Per Cent. of ♂♀.	No. of ♀♀.	No. of ♂♀.	Per Cent. of ♂♀.	No. of ♀♀.	No. of ♂♀.	Per Cent. of ♂♀.		
1–56	445	100	18.34+	529	16	2.93+	533	18	3.26+	Sept. 3, 1909, to Jan. 16, 1910.	Uncooked concentrated horse manure media, 2–5 days old. *Polytoma* and other protozoa.
57–345	2,565	92	3.46+				2,749	0	0	Jan. 16, 1910, to Nov. 15, 1911.	
57–238				1,731	8	0.45+				Jan. 16, 1910, to Mar. 6, 1911.	Cooked concentrated horse manure media, 1½–3 days old. Pure culture of *Polytoma*.

present evidence collected from observations extending over a period of about three years and including 300–500 parthenogenetic generations shows very clearly that strains exist which differ in their percentage of male-producing females.

Moreover, the two sister strains *A* and *B* which developed from the same fertilized egg differed in their longevity. Strain *B* died out from general exhaustion in the 384th parthenogenetic generation, while strain *A* is still alive in the 504th parthenogenetic generation, although in a very weak and exhausted condition.

Shull has compared some of the New York strains of *Hydatina senta* with a strain from Baltimore and has found a decided difference in the rate of production of males in the two strains. He says, "It is safe to say, therefore, that we have here two pure lines that differ from one another in a fairly constant manner, and the difference is an internal one."

SUMMARY.

1. The production of male-producing females can be partly or wholly repressed by external conditions in parthenogenetic races of *Hydatina senta*.

2. The parthenogenetic strains are shown to be distinct because under identical external conditions they differ in their power to produce male-producing females. This may indicate that they differ in their potentiality of producing male-producing females or that they differ in degree of responsiveness to the influences which cause male-producing females to be produced. The latter alternative seems more probable.

3. The two sister parthenogenetic strains developing from one fertilized egg differed in their longevity. One lived about a year longer and produced over one hundred more generations than the other.

BIOLOGICAL LABORATORY,
WESLEYAN UNIVERSITY,
MIDDLETOWN, CONN.,
January 3, 1912.

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SUPERNUMERARY CHROMOSOMES, AND SYNAPSIS IN CEUTHOPHILUS (SP.?).

N. M. STEVENS.

The species of *Ceuthophilus* which I have used in this study, I have not been able to identify. The material seems to be homogeneous, and is the only species of this genus that I have seen about Bryn Mawr. The insects were found, usually in pairs, in their burrows under stones, and were collected in October and November, 1910 and 1911. They are not abundant, and only 7 males were secured in 1910, and 5 in 1911.

METHODS.

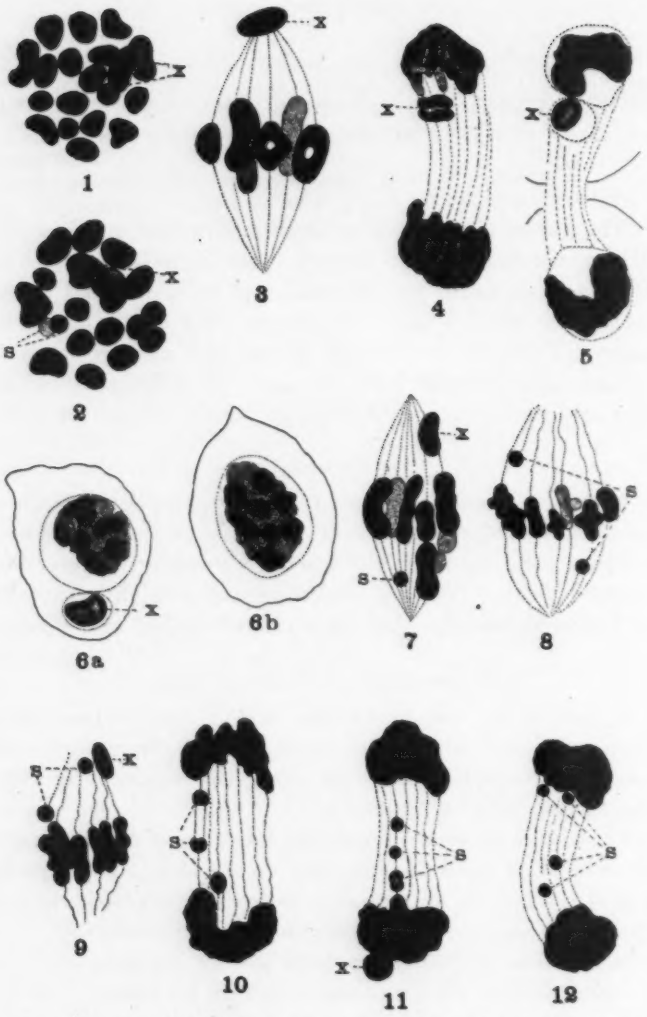
In the case of each individual a few aceto-carmine preparations were made, and the remainder of the testes fixed in Gilson's mercuro-nitric fluid or in Flemming's stronger chrom-osmic-acetic mixture. The best results were obtained from sections of Flemming material, cut 10μ thick and stained with thionin.

SUPERNUMERARY CHROMOSOMES.

In one of the 1910 insects one, and in another two supernumerary chromosomes were found. These chromosomes are readily distinguished from the other chromosomes by their smaller size and erratic behavior.

The spermatogonial chromosomes, like those of *Stenopelmatus*, are difficult to count, because they do not form a flat plate at any stage, but lie at somewhat different levels and overlap. The number is probably 37, exclusive of supernumeraries.

In the first spermatocytes there are 18 bivalents and the univalent X (19), when no supernumeraries are present (Fig. 1). Fig. 2 shows 18 bivalents, the unpaired chromosome X, and 2 supernumeraries (s). The odd chromosome X is usually found at one pole of the spindle when the other chromosomes are in metaphase (Fig. 3), but it not infrequently lags behind the others in the anaphase (Fig. 4) and is enclosed in a separate membrane



FIGS. 1–2. Metaphase of first maturation mitosis, showing $18+X$ and $18+X+2s$. (Mag. 1,500 for all figures.)

FIGS. 3–5. Metaphase and anaphases showing position of X.

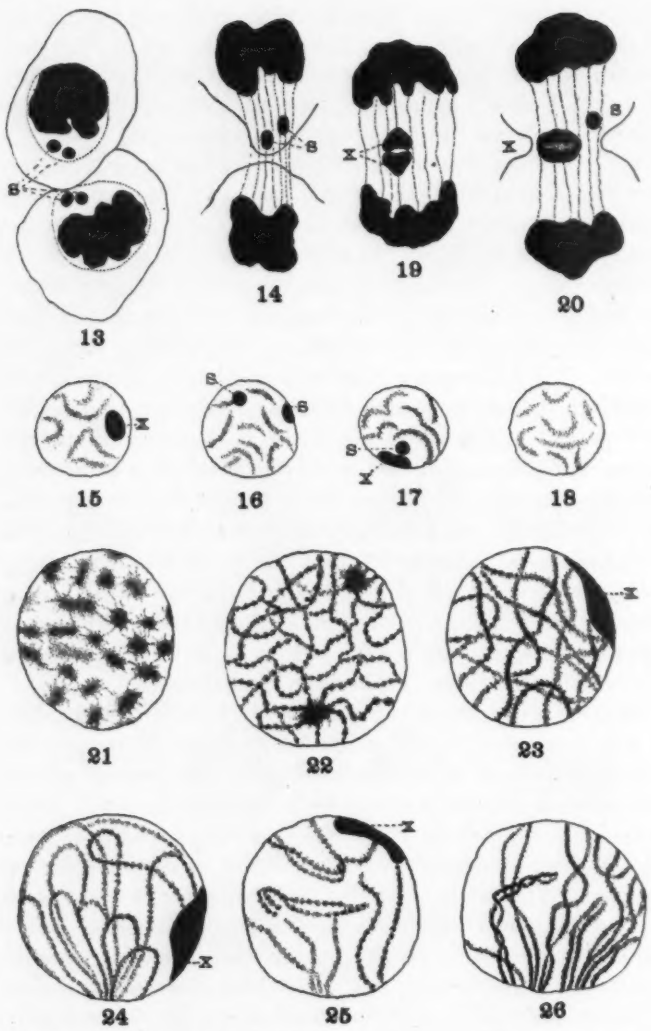
FIGS. 6*a* and *b*. Young sister spermatocytes of the second order.

FIGS. 7–12. Variations in position and division of the two supernumeraries in first spermatocytes.

in the telophase (Fig. 5). Figs. 6*a* and 6*b* are sister spermatocytes of the second order, showing *X* in a separate vesicle; this does not happen in by any means one half of the second spermatocytes, *X* being, I should say, more often included within the same nuclear membrane with the other chromosomes.

Fig. 7 is from the individual which had one supernumerary, and Figs. 8 to 14 from the one that had two. Fig. 8 also shows a less usual position of *X*, near the equatorial plate. As in the *Diabroticas* (Stevens, '08) the supernumeraries may or may not divide in the first maturation mitosis, and they may, when undivided, go to either pole of the spindle. Their position in the metaphase does not necessarily indicate whether they will divide or not. The determining factor is probably the attachment of spindle fibers from one or from both poles. The supernumeraries in Figs. 7, 8 and 9 would not divide in this mitosis, but the presence of a supernumerary in an equatorial plate or between daughter plates does not necessarily assure its division. In Fig. 10 one is divided, the other undivided, while in Figs. 11 and 12 both are divided. In the telophases shown in Figs. 13 and 14, both supernumeraries are divided in one case and neither in the other. The possible combinations of *X* and the 2 supernumeraries in the spermatids are *X*, $X + 1s$, $X + 2s$, 0, $1s$ and $2s$. Four of the possibilities are shown in Figs. 15 to 18.

As to the origin of these supernumeraries, there is little evidence in this material. In *Metapodius* Wilson ('09) discovered the probable origin of the supernumeraries in an irregular second spermatocyte mitosis in which both "idiochromosomes" went to the same pole of the spindle, and therefore to the same spermatid. The supernumeraries are thus shown to be duplicates of the smaller "idiochromosome" in *Metapodius*, or in one case of an "*m*-chromosome" ('10). In neither *Diabrotica* nor in *Ceuthophilus* is there a smaller mate for the *X* chromosome present. The three supernumeraries which have been observed in *Ceuthophilus* are of about the same size; considerably less than one half, and apparently about one fourth the size of *X*. The behavior of the supernumeraries in growth and rest stages of the nucleus indicates their probable relationship to *X*, and their behavior in mitosis, dividing only once, either in the first or the second



FIGS. 13–14. Telophases showing the supernumeraries divided (13) or undivided (14).

FIGS. 15–18. Spermated nuclei showing variations as to presence or absence of *X* and the two supernumeraries.

FIGS. 19–20. Anaphases showing unusual position of *X*.

maturation mitosis, shows that they are univalent. In *Diabrotica soror* I have considerable evidence that the supernumeraries owe their origin to a transverse and a longitudinal division of *X* ('12), and it seems probable that those of *Ceuthophilus* have had a similar origin. I have occasionally found cases where *X* seemed about to divide late in the first maturation mitosis (Figs. 19 and 20), but I have as yet no evidence of a transverse division.

Synapsis.

The material which was collected in 1911 with the hope of getting more light on the origin of the supernumeraries, proved to be favorable for a study of synapsis, or, as I should prefer to call the phenomenon, conjugation of the chromosomes. These testes were all fixed in Flemming and stained with either thionin or iron-hæmatoxylin. Thionin gave the clearest figures.

In the resting nuclei of the spermatogonia the chromosomes are either visible as separate individuals as in Fig. 21, or are more or less completely resolved into rather fine spireme threads as in Fig. 22. In some follicles one sees only such spermatogonial nuclei as in Fig. 21; in others the various cysts show various degrees of resolution into spireme threads. The former condition I should attribute to more rapid division of the spermatogonia, the time between mitoses being insufficient for complete resolution.

In the youngest spermatocytes, distinguished from the spermatogonia by the condensed condition of *X*, the spireme threads are similar to those of the spermatogonia, perhaps a little coarser. They are finely granular and more or less nodular. There is no contraction, or synizesis, stage and no complete polarization of loops to form a perfect bouquet stage. The spireme threads are usually irregularly but rather evenly distributed through the nucleus as in Fig. 23, which also shows *X* in characteristic position against the nuclear membrane. Fig. 24 shows an extreme and

Figs. 21–22. Spermatogonial nuclei, showing resolved and unresolved chromosomes.

Fig. 23. Spermatocyte nucleus before synapsis.

Fig. 24. Similar stage showing partial polarization of chromosomes.

Figs. 25–26. Stages in parasynapsis.

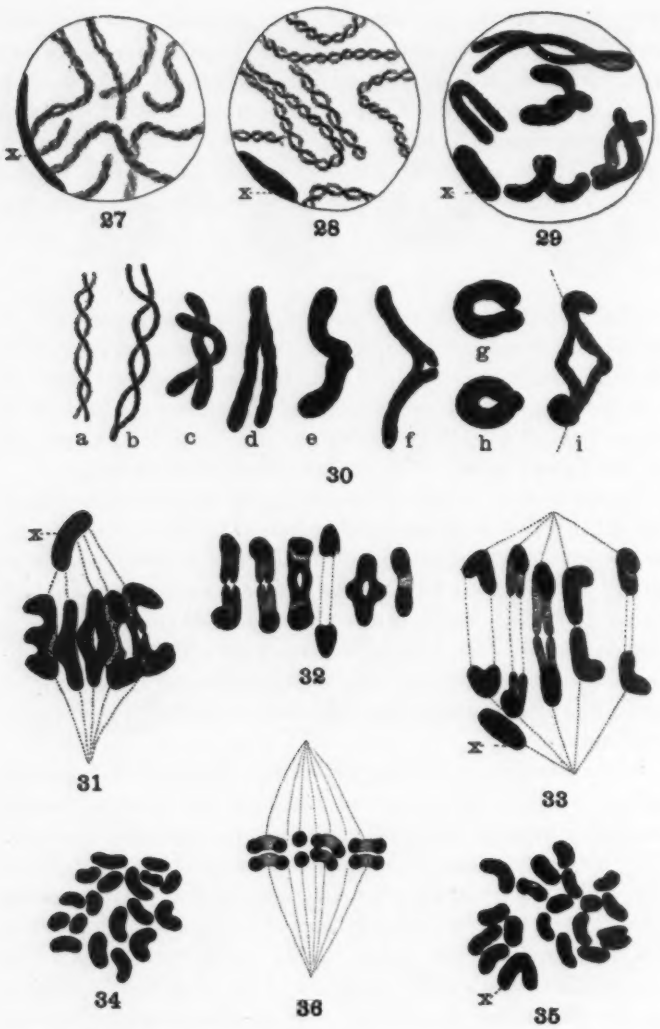


FIG. 27. Double spireme stage.

FIGS. 28–29. Prophase stages.

FIG. 30*a–i*. Various prophase (*a–g*) and metaphase (*h*, *i*) forms of the bivalent chromosomes.

FIGS. 31–32. Chromosomes in metaphase of the first maturation mitosis.

unusual case of polarization, but here only nine polarized loops are present and the other spireme threads extended in various directions through the nucleus. In some follicles there is a perfectly sharp division-line between cysts containing spermatocyte nuclei of the character of that shown in Fig. 23, and cysts in which the spireme is about twice as thick (Fig. 27). There is no evidence of telosynapsis, and also no evidence of any such longitudinal contraction of the threads as would be required to give the thicker spireme of Fig. 27. In other follicles there come in between these two stages, other cysts in which as a rule the chromatin threads are very irregularly distributed; *i. e.*, there is a conspicuous absence of the rather even spacing of the threads observed in Figs. 22 and 23. Careful inspection of such cysts reveals various stages in pairing, conjugations, or synapsis of the chromatin threads (Figs. 25 and 26). In the same cyst one often finds various stages,—only one or two pairs and the other threads single as in Fig. 25, and all stages up to one in which all of the threads are in pairs. Occasionally some of the pairs in a nucleus show as much polarization as is indicated in Fig. 26, but in the same nucleus other pairs are differently distributed. There is never such complete polarization as is shown in some of the figures of Grégoire ('10), the Schreiners ('04), Agar ('11) and others. The occurrence of various stages of parasynapsis in the same cyst, the substantial agreement in size of the chromatin threads through the stages indicated in Figs. 23–26, and the spireme of double size in the next stage, preclude the possibility of interpreting the paired condition seen in Figs. 25 and 26 as a longitudinal split. Homologous chromosomes in some way come together, and gradually twist up into a tighter and tighter rope-like strand. A casual comparison of cysts in the stage shown in Fig. 27 with the earlier stages (Figs. 23 to 26), using low powers of the microscope, simply gives the impression that here we have a spireme, or sections of a spireme, twice as thick as in the previous stages, but study of such nuclei with Zeiss 1.5 mm. and oc. 12 reveals the double and twisted condition of the strands

FIG. 33. Anaphase showing segregation of homologous chromosomes and longitudinal splitting.

FIGS. 34–36. Second maturation mitosis showing dimorphism in number (18 and 19) and equational division.

in practically every nucleus, indicating that the paired threads are at no time so thoroughly fused as even apparently to lose their identity. This is further indicated by the earliest prophase stage (Fig. 28) where the paired strands begin to untwist. The following prophase stages consist of further untwisting and longitudinal contraction of the paired homologous chromosomes. In the synapsis stage (Figs. 25 and 26) it is impossible to tell whether threads of equal length form the pairs, but in the prophase pairs this is perfectly evident (Figs. 29 and 30). The untwisting and contraction frequently proceed at different rates in different pairs in the same nucleus and in different nuclei of the same cyst, so that one can easily compare the various stages of the process and be perfectly sure that the untwisting is continuous. There is no secondary fusion of paired threads such as frequently occurs in cases where a precocious longitudinal split appears in a telosynaptic bivalent and then closes up before the rings and crosses are formed (see *Blattilla germanica*, Stevens, '05). Fig. 30 shows various stages in the formation of the definitive chromosomes of the first maturation mitosis from the parasynaptic threads of a stage a little later than that of Fig. 28. The paired chromosomes untwist and contract simultaneously. Some remain united at one end (*b* and *d*) while in other cases union of a pair at one or both ends is a secondary phenomenon and may even occur after the spindle has formed (*a* and *c*). That there is much variation in the form and size of the 18 bivalents in metaphase is shown in Figs. 3, 7, 8, 9, 30, 31, 32 and 33. The most frequent forms are rings, E's and crosses, though one or more pairs of straight rods may be found in nearly every spindle. Most of the chromosomes are attached to the spindle fibers at or near the middle of each univalent member of the pair, so that the separated chromosomes pass to the poles of the spindle in the form of V's (Figs. 31 to 33). In the case of the double rods the fibers are attached at the ends. Many of the chromosomes are partly or wholly split longitudinally in the anaphase (Fig. 33). There are of course two kinds of second spermatocyte equatorial plates containing 18 and 19 chromosomes respectively (Figs. 34 and 35), *X* appearing in the form of a large V (Fig. 35) in one half of the cells. Division of the chromosomes is here longitudinal as seen in Fig. 36.

In *Ceuthophilus* the first maturation mitosis is therefore a segregating division of the previously paired and united homologous univalent chromosomes, while the second mitosis is as clearly an equational division of all of the univalent chromosomes including *X*.

Discussion.

In an earlier study ('05) of the spermatogenesis of two other species of Orthoptera, *Blattella* (*Blatta*) *germanica* and *Stenopelmatus* (sp.?), I found what seemed to be good evidence of telosynapsis ('05, Pl. II., Figs. 55, 56, 58, 59, 62, 63, 64, and Pl. III., Figs. 108 to 115). That material I have reviewed and compared with the *Ceuthophilus* preparations, and I find no such evidence of parasynapsis in either of them. Naturally I expected to find telosynapsis in *Ceuthophilus*, and was surprised on working backward from the maturation mitoses to find no evidence of telosynapsis outside of the late prophases, and abundant evidence of parasynapsis in the young spermatocytes at a stage where synizesis is frequently found in other material.

A recent review of the literature on conjugation of chromosomes has only strengthened my previous conviction, based on my own experience with the spermatogenesis of a variety of forms, that the phenomenon is one which varies greatly in different groups of organisms, and even in different species of the same genus, or different sexes of the same species (*Sagitta*, Stevens '03, '05; *Bufo*, King '07, '08). Indeed I should not be surprised if the range of variation should prove to extend from (*a*) cases where there is nothing that could be called conjugation, but merely such a pairing, without contact even, as will secure segregation of homologous maternal and paternal chromosomes to different daughter cells, through (*b*) an intermediate condition of telosynapsis and less intimate parasynapsis, to (*c*) cases where homologous chromosomes are so completely fused in parasynapsis that it is impossible to tell whether the resulting chromosomes which are segregated in mitosis are identical with those that went into synapsis or not; and the variation may extend to cases which may give further support to Janssens' chiasma theory ('09) or to Morgan's modification of it ('11) in which homologous chromosomes are supposed to be twisted tightly together in

parasynapsis and split across the twists in preparation for mitosis, giving daughter chromsomes which contain both maternal and paternal chromatin.

In *Ceuthophilus* the parasynapsis stage of Fig. 27 is intimate enough and long enough to favor the supposition that it is a true conjugation involving exchange of material particles or of chemical substances (genes), but there is no evidence of any splitting of Morgan's chiasma type. All of the evidence indicates that homologous paternal and maternal chromosomes twist together in parasynapsis and untwist in the prophase of the first maturation mitosis. In the flies and mosquitoes (Stevens, '08, '10, '11) we have examples of even more pronounced parasynapsis than in *Ceuthophilus*, but so far as I have seen, the indications are that the chromosome pairs twist up in synapsis and untwist in prophase much as in *Ceuthophilus; i. e.*, an opportunity for interchange of genes between homologous maternal and paternal chromosomes is furnished by the observed phenomena of parasynapsis in these forms, but no evidence of such a chiasma type of splitting after synapsis as is suggested by Morgan ('11) to account for the results of his breeding experiments with *Drosophila*. Such an exchange of parts of chromosomes as that described by Janssens ('09) might of course occur without being detected, at almost any point in the process of twisting or untwisting of the pairs, since the time element is not determinable in fixed preparations.

Moreover, it seems to me that, in view of the great range of variation in the phenomena of conjugation and segregation of the chromosome in the maturation of germ cells, cytological evidence from one form cannot safely be taken to serve as the basis of a theory or hypothesis to account for the experimental results on another form, but cytological and experimental work on the same form must go hand in hand, in order that any safe conclusions may be drawn from the results.

There seems to be no question but that synapsis, or conjugation of the chromosomes is the most difficult phenomenon connected with the maturation of the germ cells, to interpret correctly, and doubtless earlier parasynaptic stages have been overlooked in some cases where telosynapsis alone has been described in con-

nection with the mitotic stages of maturation, but it seems to me quite unlikely that synapsis in all organisms follows one method; and, moreover, I believe that the variations in method of synapsis and intimacy of union of homologous chromosomes in different forms will be found to be directly connected with variations in methods of inheritance of unit characters, especially in relation to interchange or lack of interchange of maternal and paternal genes. If this is true we should expect to find more cases of complete coupling of unit characters where telosynapsis or no real synapsis occurs. If parasynapsis is an adaptation to secure interchange of genes, we should expect to find cases of telosynapsis followed by parasynapsis, as indicated, but not certainly demonstrated in the guinea-pig (Stevens, '11, Figs. 9, 10, 11). In my studies on spermatogenesis of the Coleoptera ('05, '06, '08, '09) I found evidence of telosynapsis in several cases and no evidence of parasynapsis, but this was only an incidental matter at the time, and of interest merely in relation to the segregation of whole chromosomes in the maturation mitoses. It is my intention to reëxamine all of my Coleoptera and Diptera material with reference to the questions whether parasynapsis occurs in the Coleoptera, and whether the Diptera show any evidence of Janssens' chiasma types of synapsis.

BRYN MAWR COLLEGE,
January 1, 1912.

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FURTHER OBSERVATIONS ON SUPERNUMERARY CHROMOSOMES, AND SEX RATIOS IN DIABROTICA SOROR.

N. M. STEVENS.

Supernumerary Chromosomes.

In the summer of 1910 while I was enjoying the privileges and hospitality of the Marine Biological Laboratory at La Jolla, California, I took advantage of the opportunity to study the male germ cells of *Diabrotica soror* from a new locality. Having previously ('08) found supernumerary chromosomes varying in number from one to five in about 50 per cent. of the male individuals of *Diabrotica soror* at Mountain View, California, and *Diabrotica 12-punctata* at Bryn Mawr, Pa., I was interested to see whether supernumeraries would be found in the same proportion in a third locality.

Testes from a hundred individuals were studied in aceto-carmine preparations. The greater part of the material was collected in a corn-field in the open country between La Jolla and the new laboratory which is two miles north of the town. A few were obtained from a rose-garden in La Jolla and one lot of 68 males and females was collected for me by Miss Myrtle Johnson on corn in a garden at National City, just south of San Diego. Individual records were kept for each lot, but the conditions with respect to number of supernumeraries proved to be about the same for the three collecting grounds.

To my surprise I found supernumeraries scarce. In the first 25 males examined, 21 had no supernumeraries and 4 one; while out of the first 25 examined the same summer at Mountain View 15 had no supernumerary, 7 one and 3 two; and in the first 25 at Mountain View in 1909, there were 13 with no supernumerary, 9 with one, 2 with two and 1 with three. In the La Jolla material the 89th individual was reached before a case of two supernumeraries was met with, and in the first 100 males 79 had no supernumerary, 20 one, and 1 two. The follow-

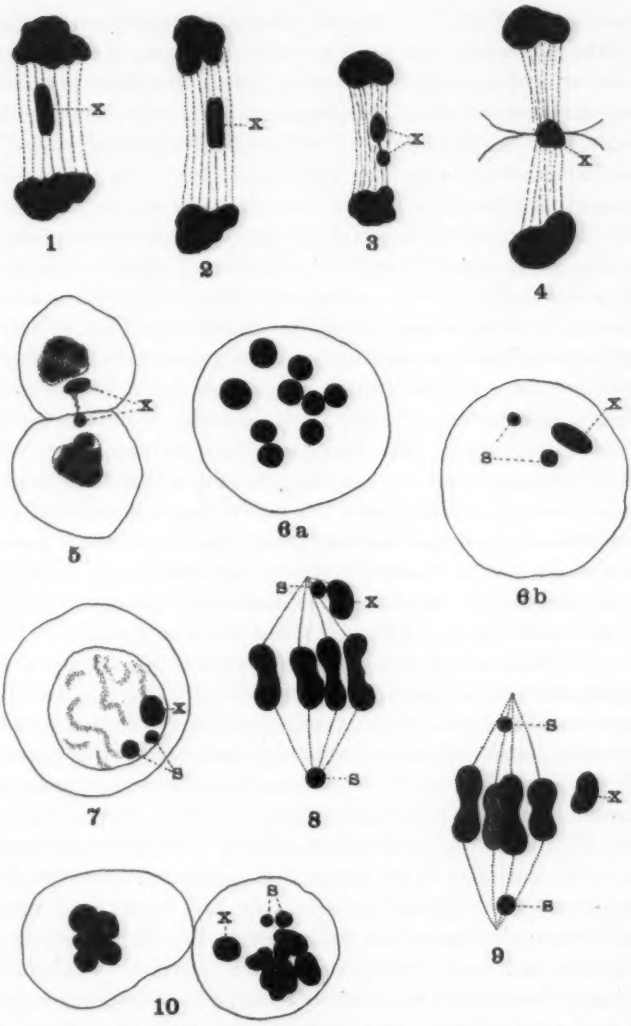
ing table shows the per cent. of supernumeraries in the two species different years and in different localities.

Number of Supernumeraries.	0	1	2	3	4	5
D. sor., Mt. V., '07, June 23–Aug. 7.	51	35	11	2	1	
D. 12–p., B-M., '07, Oct. 3–9.	48	33	15	3	1	
D. s., Mt. V., '09, July 10–Aug. 12. .	43	44	10	3		
D. s., Mt. V., '09, Aug. 21–Sept. 15.	46	38	10	4		2
D. s., Mt. V., '10, July 28–Sept. 1. . .	52	29	16	3		
D. s., La J., '10, June 17–July 4. . . .	79	20	1			

As I had never seen any signs of degeneration of the supernumeraries, the natural interpretation of their infrequency at La Jolla would seem to be either that they had originated here more recently, or that they had originally appeared in fewer individuals in this locality.

The behavior of supernumeraries in all cases where they have been shown to occur at once classes them with the heterochromosomes, and in *Metapodius* Wilson ('09) has shown that they have probably originated in an irregular second maturation mitosis in which both idiochromosomes went to one pole of the spindle instead of separating. He therefore regards the supernumeraries in *Metapodius* as duplicates of the smaller idiochromosome. In 1908 I suggested that there might be two varieties of *Diabrotica soror* and also of *D. 12-punctata*, one having only the odd heterochromosome and the other an unequal pair, and that hybridization might have given rise to the supernumeraries with their peculiar behavior, dividing sometimes in one sometimes in the other maturation mitosis. I have, however, been able to find no evidence in favor of this view. In 1910 I studied carefully the testes of many individuals where no supernumeraries were present, seeking some clue to the origin of these chromosomes.

As a rule the odd chromosome X appears near one pole of the spindle in the metaphase of the first maturation mitosis, but I had always noticed that occasionally X is in or near the equatorial plate, and in some individuals this is quite common. At La Jolla I found two spindles in which X was between the daughter plates in the anaphase, and stretched out lengthwise (Figs. 1 and 2). In one of these cases (Fig. 2) X was split so



FIGS. 1–5. Anaphases of first maturation mitosis, showing abnormal position and transverse division of *X*. (Mag. 1,500 for all figures.)

FIGS. 6*a* and *b*. Metaphase of first maturation mitosis, showing two supernumeraries (*s*) unequal in size.

FIGS. 7–10. Other stages from same testis showing behavior of *X*, and the two supernumeraries.

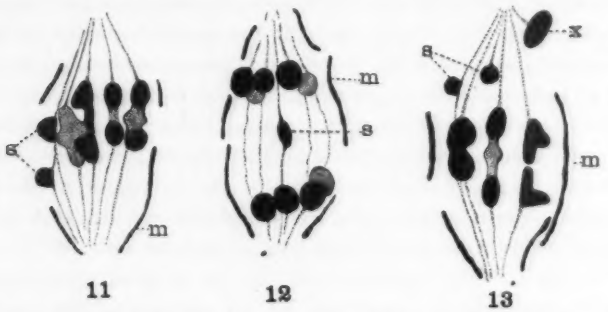
that it was certain that it was in a position such that it might divide transversely, but I was not able to find any cases of actual transverse division of *X*. Later at Mountain View I did find two anaphases where *X* appeared to have divided transversely and unequally (Figs. 3 and 5) and one in which *X* was caught in the cell plate between the daughter cells (Fig. 4). Now the supernumeraries are usually very uniform in size and certainly less than one half the sixe of *X*. I have one individual noted as having an unusually large supernumerary, about one-half as large as *X*, and a few cases where an unusually small one occurs. One of the latter cases is shown in '08, Pl. III., Figs. 76 to 78. From the evidence now at hand I should infer that the probable origin of the supernumeraries in the *Diabroticas* has been an occasional transverse division of *X* followed by a longitudinal division of the two parts. Evidently the transverse division has usually been an equal one, but that it may be unequal is shown by Figs. 3 and 5, and the rather rare occurrence of unusually large and unusually small supernumeraries. Figs. 6 to 10 are from a male captured at Mountain View, July 29, 1910. Here we have a large and a small supernumerary in the same individual. In the metaphase (6*a* and 6*b*) *X* and the two supernumeraries were all near one pole of the spindle, while in Figs. 8 and 9 the supernumeraries are at opposite poles and in Fig. 9, *X* is near the equatorial plate. In Fig. 10, *X* and both supernumeraries have gone undivided to one second spermatocyte. No cases of the division of either supernumerary in the first maturation mitosis were found in this individual.

In *Metapodius* Wilson found no somatic variations corresponding to the variation in the number of supernumeraries. In fact the insects with *X* alone, *X* and *Y*, or *X*, *Y* and 1 to 6 supernumeraries are described as indistinguishable. These species of *Diabrotica* are very variable in size, and in regard to size and fusion of the 12 black spots on the elytra, but as I showed in 1908 there is no significant correlation between these somatic variations and the presence or absence or number of supernumeraries ('08, Tables I. and II., and p. 465, text). In *Metapodius* the indications are that the chromosome *Y* is of no hereditary value. and the supernumeraries, as duplicates of *Y* would not be ex-

pected to affect the somatic characteristics of the insects. If, however, the supernumeraries of *Diabrotica* come originally from different regions of *X*, there would seem to be no reason why they should not bear functional genes for sex and other characters. The male always contains *X* so far as my experience goes (over 700 males), but one would suppose, if the supernumeraries are functional in heredity, that one *X* and a supernumerary might frequently determine the development of a female, and if so there should be males without *X*, but with a supernumerary in its place. It may, of course, be true that the abnormal division of *X* producing supernumeraries in itself indicates a degenerate or non-functional condition of that particular *X* chromosome, and therefore of its progeny—the resulting supernumeraries. This would fall in line with Schleip's ('11) suggestion in regard to the rejected *X* chromosome in the spermatogenesis of the hermaphrodite generation of *Angiostomum nigrovenosum*, that it had already become non-functional at an earlier stage, whence its later behavior. It is exceedingly desirable that the female sex cells of these *Diabroticas* should be studied, but I have not been able to get any favorable mitoses in the adults, or to secure larvæ or pupæ from the soil or roots of plants on which they live. Several attempts to breed them have given no results.

The testes of 12 males each having one supernumerary were studied from the point of view of the division of the supernumeraries in the first maturation mitosis. All anaphases and metaphases in each preparation were examined and all cases where it was possible to determine the position and behavior of the supernumerary recorded. In the metaphase the supernumerary was in the equatorial plate in 54.1 per cent. of 901 cases and out of the plate—nearer one pole of the spindle—in 45.9 per cent. Apparently the supernumeraries, when they divide, do so later than the bivalent chromosomes, so all anaphases were examined on this point. In 55.6 per cent. of the anaphases found in the 12 testes, the supernumerary was between the daughter plates, and in 44 per cent. it was dividing or divided. Here the 56.6 per cent. corresponds closely with the 54.1 per cent. in the equatorial plates in the metaphase, and the 44.4 per cent. outside of the daughter plates in anaphases comes

very near the 45.9 per cent. out of the equatorial plates in metaphase. The division of supernumeraries or their failure to divide in the first maturation mitosis seems to be a matter of chance, depending on their position in the spindle in the prophase and on the attachment of spindle fibers from one or from both poles of the spindle. In Fig. 11 both supernumeraries are connected by fibers with both poles, in Fig. 12 the *s*-chromosome is connected with both poles and is about to divide, and in Fig. 13 one *s*-chromosome is connected with both poles and will later divide, while the other will go undivided to the upper pole of the spindle and therefore to one second spermatocyte. The behavior of the other chromosomes indicates a more or less definite attachment point for the spindle fibers, near the middle of the chromo-



11 12 13

FIG. 11. Spindle showing two supernumeraries (*s*), each attached to spindle fibers from both poles. Mag. 2,000.

FIG. 12. Anaphase showing supernumerary (*s*) about to divide.

FIG. 13. Metaphase showing *X*, a supernumerary (*s*) attached to one spindle fiber, and another (*s*) attached to two. *m* = mitochondria.

some in both spermatogonial and spermatocyte mitosis (Figs. 11 and 13). The supernumeraries seem to be able to make connections with both poles in most cases if they are in or near the equatorial plate in late prophase stages.

SEX RATIOS.

The sex ratios in *Diabrotica soror* and *Diabrotica 12-punctata* have shown very peculiar variations. In studying the male germ cells of *D. soror* in 1907 I made no note of the number of females found in random collections, but in dissecting *D. 12-*

punctata in October, 1907, I found more than two males to one female,—in one lot 58 males to 25 females. In 1909 the number of males and females was noted for each lot dissected. Between July 10 and August 12, 107 males and 102 females were counted in random collections from two neighboring gardens, but it was noticed that the ratios in the two gardens were quite different. In garden *A* there were 58 males to 26 females; in garden *B*, 49 males to 76 females. A second lot from garden *A* collected between August 21 and September 15 gave 101 males to 24 females. The percentage of females in garden *A*, first lot, was 30.9, second lot 19.2, average 23.9, and for garden *B* 60.8.

At La Jolla in 1910 the ratios ran more evenly.

	♂	♀
La Jolla, June 17 and 18	25	16
National City, June 22	34	34
La Jolla, June 28	14	19
La Jolla, July 1	14	25
La Jolla, July 4	20	27
	107	121

At Mountain View again the ratios were peculiar. Five random collections in Garden *A* gave 100 males to 20 females, and two other later collections 76 males to 18 females. Only a few were collected from garden *B* giving 12 males to 6 females. In 1911 mixed lots from both gardens gave more males than females, 61 : 47. These were recorded incidentally while fixing a lot of testes for sections. By referring to the table on page 232, it will be seen that the number of supernumeraries runs about the same for the first 100 in 1909, about one half of which came from each garden (*A* 51 and *B* 49), and for the second 100, all of which came from garden *A*. It therefore seems unlikely that the supernumeraries have anything to do with the difference in sex ratios in the two gardens. The soil in garden *A* is harder in summer—more adobe in it—and less thoroughly cultivated than *B*. Two possibilities are suggested in this connection: (*a*) The males may be more successful in pupating and escaping from the hard soil than the females or (*b*) few of either sex may emerge from the hard soil in garden *A*, and the males may be better flyers and so come in larger numbers from other neighboring gardens. The latter is regarded as more probable.

The Bryn Mawr *Diabroticas* of 1907 were all collected on a large clump of golden rod in a pasture that had not been cultivated for many years, and they may have come out of the ground in the immediate neighborhood or from more recently cultivated fields near by.

These erratic sex ratios are probably merely another example of the interference of external conditions in what would otherwise be an equality of sexes, or in other words a shifting of normally equal sex ratios, or partial exclusion of one sex by peculiarities in the environment. The collections were all random in the sense that all the individuals that could be found were collected each time.

BRYN MAWR COLLEGE,
January 3, 1912.

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THE RELATION OF THE FIRST CLEAVAGE PLANE TO THE ENTRANCE POINT OF THE SPERM.*

ERNEST E. JUST.

During the summer of 1911 at the Marine Biological Laboratory under the direction of Professor Frank R. Lillie, I was engaged in the study of the eggs of *Nereis* of certain cytological problems the results of which will appear later. The question of the relation of the entrance-point of the sperm and the first cleavage plane occurred to me. A very pretty method made possible in a satisfactory fashion the determination of this relation the results of which this paper embodies. I here take this opportunity to express my thanks and sense of gratitude to Professor Lillie for his inspiring interest in the work of which this is a part.

Material and Methods.

The eggs of *Nereis* when shed are irregular in shape due to pressure while in the body of the female. They soon fill out in the sea water, measuring about 100 μ equatorially and somewhat less in a polar direction. There is, however, a great deal of individual size variation in the eggs of a given female The eggs are almost transparent, colored a pale green by numerous deutoplasm spherules distributed throughout the endoplasm; around the equator is an irregular double girdle of 14 to 22 oil drops (Fig. 1). In polar view the large germinal vesicle appears to be in the center of the egg. It is, however, slightly elongated in the polar direction. The polarity of the ovocyte is, therefore, expressed by the polar flattening already mentioned, the position of the oil drops, and the form of the nucleus.

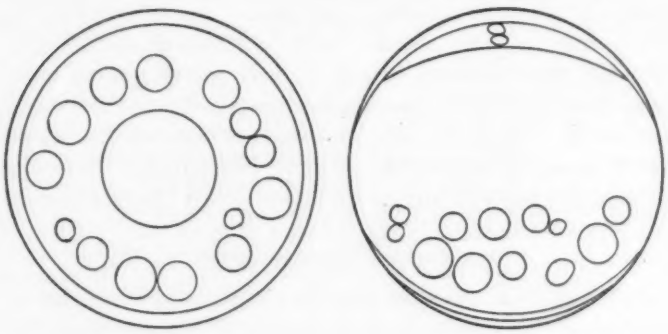
As has been shown (Lillie, '11) there are not two membranes in the unfertilized egg of *Nereis*, but rather a single vitelline membrane external to the radially striated cortical layer ("zona radiata," Wilson) of the egg. The ovocyte remains thus with

*All drawings, of living eggs, made with the aid of a camera lucida.

nucleus intact until inseminated or otherwise stimulated—as for instance, by squirting forcibly through a pipette.

Two or three minutes after insemination, a jelly is rhythmically extruded from the cortical protoplasm. In ten minutes the germinal vesicle breaks down, development is initiated.

Males and females captured in the evening while swimming at the surface of Eel Pond were kept in separate dishes until morning when they were transferred to fresh clean sea-water. To get an abundance of eggs and of sperm for an experiment, it was merely necessary to cut open a female and a male. The cut animals



1 2

FIG. 1. Egg of *Nereis* at time of insemination; polar view.

FIG. 2. Maturation stage; second membrane formed; oil drops at vegetative pole.

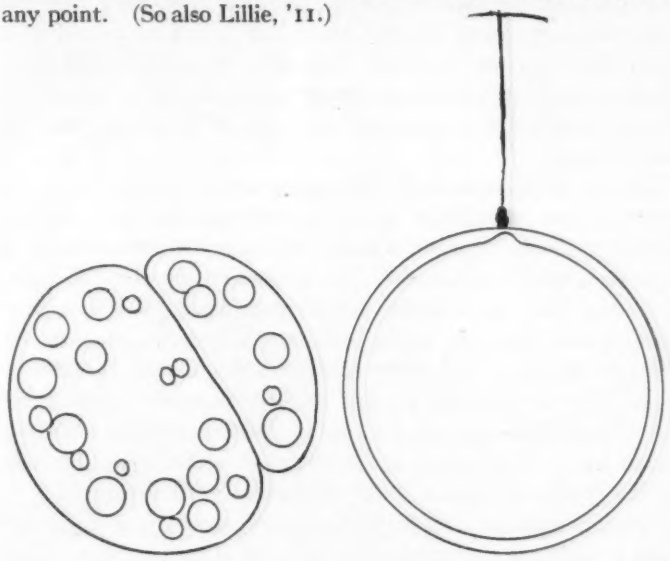
were removed from the dishes at once; moreover, every other precaution was taken to avoid abnormalities superinduced through toxic influences, mechanical shock, etc. In several watch glasses of sea water in which India ink had been ground up eggs were put together with a minute quantity of sea water containing very few spermatozoa. The time of insemination was noted and the numbered dishes observed to the second cleavage. This method was varied somewhat as I shall later note.

Ringed slides also were used; eggs placed on these in sea water and ink were inseminated. Sometimes a cover slip was placed on the eggs. Finally, for the later observations a very few eggs were placed on slides and the cover slips supported with glass rods.

Observations.

Outline of Development to First Cleavage.

Eggs in sea-water in which India ink has been previously ground up show clearly the formation of the jelly, the formation of the fertilization cone, and the entrance of the spermatozoön. A streak of ink points like a dagger or an exclamation point to the entrance cone above which on the membrane the spermatozoön is attached (Fig. 4). This "exclamation point" is an aid quickly to determine in a large number of eggs the relation of the sperm entrance-point. The spermatozoön enters the egg at any point. (So also Lillie, '11.)



3 4

Fig. 3. First cleavage.

Fig. 4. Cone and indicator formation, 15 minutes after insemination. Outer line marks boundary of jelly.

This ink "exclamation point," or "sperm indicator" as I shall call it, is a very interesting and striking formation worthy of detailed study. With me, however, the interest lay not so much in the formation of this indicator as in its availability to help answer the question: What is the relation of the sperm entrance

point to the first cleavage plane? I here, therefore, give only as much of an outline of its formation and of the development of the egg to the time of first cleavage as will suffice to render intelligible the subsequent record of observations.

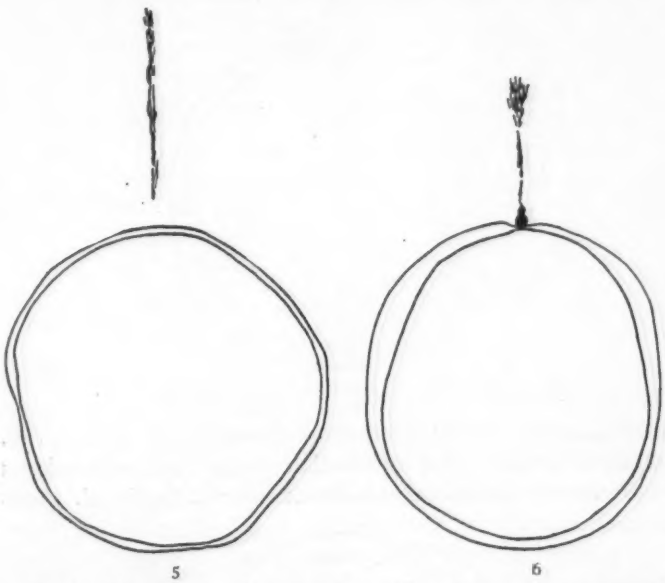
Almost at the moment the spermatozoön touches the egg membrane, the contents of the cortical layer begin to flow out as a viscid transparent substance of the same refractive index as water, leaving only radiating lines across the space (perivitelline space) between protoplasm and membrane which represent the walls of the emptied alveoli. This jelly in its flow carries the ink from the periphery of the egg so that between each egg and the surrounding ink is a clear space. This outflow of jelly may last for fifteen minutes. The jelly forms about the egg a layer everywhere continuous except along the tail of the sperm which thus forms a canal that increases in length as the jelly area widens.

Below the spermatozoön, the protoplasm of the egg begins to form a cone at thirteen to fifteen minutes after insemination which gradually increases in height until it reaches the membrane and then slowly retrogresses. With this retrogression, the membrane at this point sinks; in this depression lies the sperm. During this behavior, as the jelly area widens, the canal in the jelly in which the tail of the sperm lies fills in with particles of ink. This process is a gradual one, the indicator reaching its maximum of development fifteen to twenty minutes after insemination. The indicator, therefore, is formed along the tail of the sperm and points to the entrance-point of the sperm.

Twenty minutes after insemination, the spermatozoön may be seen attached to the membrane at the end of the indicator. The perivitelline space now becomes slight. The egg "assumes an amœboid appearance" (Wilson), changing its shape and becoming very irregular. The sperm cannot be seen readily (Fig. 5). About forty minutes after insemination the egg becomes spherical again. The sperm is easily visible on the membrane which is more widely separated from the protoplasm by the perivitelline space.

This condition is of short duration for the egg begins another series of changes. The membrane appears everywhere equi-

distant from the egg except at the point of sperm attachment where it is nearer the membrane. Then gradually to the right and left of the point of sperm attachment the perivitelline space becomes greater; the egg elongates along a line through the point of sperm attachment (Fig. 6). With the disappearance of the sperm head within the egg (about fifty minutes after in-



5 6

FIG. 5. After retraction of cone; membrane close to the egg. Sperm apparently in the egg.

FIG. 6. Two minutes before sperm is engulfed.

semination) this elongated appearance is lost (Fig. 7): the egg rounds out. The egg flattens at the animal pole (Fig. 8) and the polar bodies are given off from a clear apparently yolk-free region of the flattened pole (Fig. 2). Some little time later the first cleavage furrow appears and the egg is divided unequally (Fig. 3).

The observations on the relation of this cleavage to the entrance-point of the sperm will be considered under three heads corresponding to the methods used.

Watch Glass Series.

A female was opened at 9:58, a male at 10:00. In five watch glasses of india ink ground up in sea-water eggs and sperm were mixed at intervals of two minutes. At 10:10 a few eggs were

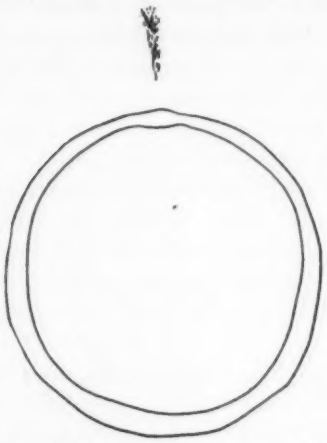


FIG. 7. Just after disappearance of sperm within the egg.

inseminated in the ink solution on an uncovered slide (no. 6). About two minutes after an insemination the jelly began to form; in fifteen minutes the sperm indicator was well developed. Eggs

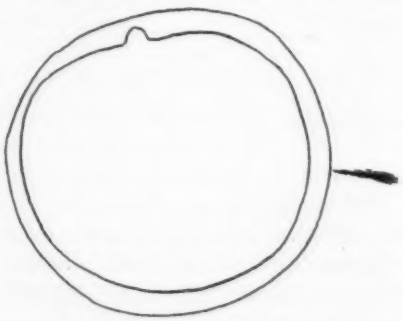


FIG. 8. First polar body forming.

inseminated at 10:15 in a watch glass (no. 7) were washed at 10:30: that is, when the indicator had reached its maximum of development.

The dishes (nos. 1 to 5) and the slide (no. 6) were examined as the first cleavage furrow appeared. In 95 per cent. of the eggs the first cleavage plane passed through the point of sperm entrance (Fig. 9). Dish no. 7 showed, on the other hand, that in only 50 per cent. of the eggs the first cleavage furrow passed through the point of sperm entrance.

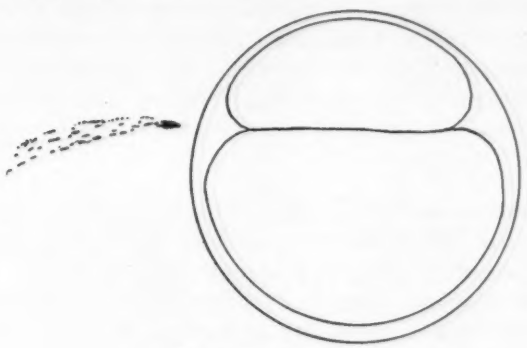


FIG. 9. First cleavage.

At 2:45 p.m. of the same day, eggs were inseminated in watch glass no. 8. Examination revealed that the first cleavage plane passed through the point of entrance in 80 per cent. of eggs. Eggs transferred from india ink and sea-water to clean fresh sea-water twenty to thirty minutes after insemination showed 60 per cent. of first cleavages through the point of entrance.

A summary of the results of Experiments 1 to 8 is as follows:

Nos. 1–5 inseminated in watch glass, not washed, showed first cleavage through entrance point, 95 per cent.

No. 6 inseminated on slide glass, not washed, showed first cleavage through entrance point, 95 per cent.

No. 7 inseminated in watch glass, washed, showed first cleavage through entrance point, 50 per cent.

No. 8 inseminated in watch glass, not washed, showed first cleavage through entrance point, 80 per cent.

No. 9 inseminated in watch glass, transferred to slide, showed first cleavage through entrance point, 60 per cent.

That the ink is not toxic to the eggs and, therefore, does not inhibit cleavage I was able to prove by inseminating at the same time two dishes of eggs, one with ink and one without; develop-

ment in both went on at the same rate and in perfectly normal fashion. I concluded, therefore, that it was not necessary to wash the eggs. Also, I found later that the eggs were often too greatly crowded and that it was hard to make counts unless the eggs were in a single layer. A trial made with very few eggs unwashed in four watch glasses gave the following result (actual numbers are given):

FIRST CLEAVAGE PLANE.

Number.	Through Entrance Point.	Not Through Entrance Point.
1	8	2
2	16	4
3	10	1
4	12	3

To what extent the eggs might rotate in the jelly was yet to be determined. It was absolutely necessary that the relation of the indicator and the sperm entrance-point remain constant; otherwise, the indicator would prove a very pretty but useless phenomenon. Could it be possible for two spermatozoa to reach the egg and the indicator to form along one sperm and not the other? How would such an egg cleave? These points were next to be determined.

I found, first, that the position of the indicator could be altered through tilting the watch glass, for the eggs would rotate in the jelly—especially when they lay on the side. I found later that the eggs are most liable to rotation after the sperm has disappeared. This might easily prove a serious source of error. Secondly, I demonstrated in several experiments that polyspermic eggs are not apt to cleave. (Professor Lillie has obtained the same results.) But with fairly dilute sperm and sea water, polyspermy, which merely cuts down the number of cleaving eggs, may be avoided.

In this connection it will be interesting to note the results obtained with old eggs and sperm. On July 30 eggs from a female captured in the evening of July 28 were used with fresh sperm—of a male captured in the evening of July 29. These eggs proved very susceptible to polyspermy. This proved true in other trials. These eggs if they segmented at all showed sixty per cent. of first cleavages through the entrance-point of the

sperm. In general, eggs that have stood in sea water for some time after leaving the female, show a low per cent. of cleavages through the entrance-point. Five hours after leaving the female eggs fail to develop on insemination.

These results seem to indicate that the first cleavage tends to pass through the sperm entrance point—*i. e.*, through the point at the end of the indicator—if the eggs be fresh, undisturbed and fertilized with a single sperm. Why then do some first cleavages fail to pass through this point? During this time a number of experiments made by day and often at night immediately after the capture of the animals showed essentially the same proportions.

Ringed Slide Series.

It was stated above, it will be remembered, that the egg tends to lie with either pole uppermost. If, however, the eggs are not disturbed those that settle on the side will so remain. The eggs are accessible to sperm at any point if not under pressure as at no time in this study they were. The first cleavage always cuts through the animal pole near the polar bodies. Obviously then, the question of the relation of the first cleavage plane to the entrance-spot of the sperm cannot be settled by the cleavage of those eggs in which the spermatozoa enter either at the point below which the polar bodies are extruded or at a spot 180° from this point.

In the next trial with very few eggs on ringed slides, those eggs in which the sperm indicator pointed either to the polar bodies or to a point 180° from the polar bodies were not counted. This trial resulted as follows:

FIRST CLEAVAGE PLANE.

Number.	Through Entrance Point.	Not Through Entrance Point.
1	14	4
2	6	4
3	20	9

Other experiments with ringed slides showed about the same proportions.

For fear that the ringed slides were toxic owing to the vaseline used they were abandoned and slides with cover slips supported

by glass rods as well as the open watch glass were used throughout the next series of observations.

Slides with Glass Support for Cover Slip.

Four or five eggs on a slide were watched continuously through the first cleavage, the indicator used merely to point out quickly the point on the membrane where the sperm was attached. Very few sperm were used in these observations, obtained through diluting several times the water which contained them. These observations were repeatedly made at night and at different times during the day. Some of the eggs failed to show the indicator and to develop. In all that segmented, *the first cleavage plane passed either directly through the entrance-point of the sperm*

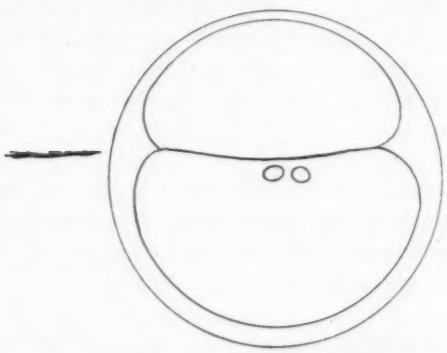


FIG. 10. First cleavage.

or a degree or so from it, with the indicator parallel to the cleavage furrow (Fig. 10). It is possible, as stated above, to keep the spermatozoön in view after the amœboid stage until it disappears within the egg. The middle piece is left without. With the aid of the middle piece, the character of the membrane at the entrance point (Fig. 7), and the oil drops near, it is possible absolutely to hold in view the exact spot at which the sperm was engulfed.

At intervals of two to three minutes, seven slides with very few eggs on each were prepared. Sperm was added and after a minute the eggs covered and every precaution taken to avoid

disturbance. In the sixty eggs counted the first cleavage furrow passed through the sperm entrance-point in every case. In some cases the indicator appeared to be at right angles to the furrow but in all such it proved to be *above* the egg and ended in the cleavage plane (Fig. 11). This was Sunday, August 20. The laboratory was quiet, the temperature conditions favorable. The results of August 23, 24 and 27 are similar. Camera sketches

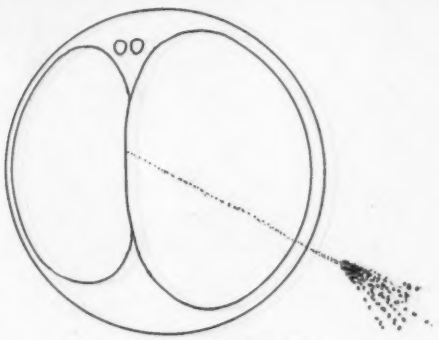


FIG. 11. First cleavage.

were made of these eggs. Often I asked an investigator, who did not know the purport of the experiments, to make the sketches; the indicator without doubt was above the egg and pointed to the cleavage furrow.

DISCUSSION.

The first cleavage plane usually coincides with the median plane of the future animal in the frog's egg, according to Roux, Newport, Pflüger and Morgan. In the squid's egg also, according to Watasé, the first cleavage plane falls in with the median plane of the embryo. Agassiz and Whitman ('84) noted a like coincidence in the teleost egg; and Van Beneden and Julin, Castle ('96) and Conklin ('05) found that the first cleavage plane marks the long axis of the embryo in the ascidian egg.[1]

[1] According to Harper, the sperm enters the pigeon's egg previous to the egg's entrance into the oviduct. He believes that the sperm must enter as soon as the disc is exposed through rupture of the follicular wall. In the pigeon's egg the sperm entrance is more or less localized. According to his figure, the first cleavage plane makes an angle of 45° with the long axis of the embryo. As we know from other researches, the long axis of the embryo is similarly placed in the egg.

But there are other eggs in which the future median plane does not fall in the plane of the first cleavage. In *Nereis* (Wilson, '92) the *second* cleavage plane, although it does not divide the animal into "equal halves," coincides with the long axis. So in *Crepidula*, the first cleavage plane is at right angles to the future median plane (Conklin, '97). In the newt (Jordan, '93) the case is the same. In *Chætopterus* (Lillie, '06) the axis of the first cleavage spindle lies in the longitudinal axis of the embryo.

There is a third group of eggs in which coincidence with any cleavage plane is wanting This is true of the egg of *Amia* (Whitman and Eycleshymer, '97), of the toadfish (Clapp, '91), and of certain amphibians (Jordan and Eycleshymer, '94), to name a few. And yet in most of these eggs the symmetry and the bilaterality of the cleavage may be sharply marked.

In the frog's egg the first cleavage plane usually and the median plane of the embryo always (*Rana fusca*) pass through the entrance point of the sperm (Roux, '85; Schulze, '99; Brachet).

In the egg of *Toxopneustes* (Wilson, '95) the first cleavage plane passes through the entrance-point of the sperm, "in the great majority of cases, at least." This plane of cleavage coincides with the transverse diameter of the embryo (Driesch).

In the ascidian egg, the belief of Castle ('96) is that the first cleavage plane cuts through the entrance-point of the sperm. Conklin ('05) says that there is no question but that the first cleavage plane is through the copulation path of the germ nuclei. And indeed his figures show very beautifully that this is actually the case.

If now we grant that in the egg of the frog and of *Toxopneustes* as in the egg of *Nereis* and of the ascidian the first cleavage plane is determined by the copulation-path, or the entrance-point, of the sperm we have this interesting conclusion: The first cleavage plane in eggs whose cleavages have different values and different relations to the future long axes of the embryos is determined by the entrance of the sperm. While the sperm entrance determines the first cleavage, the first cleavage does not in all of these forms coincide with the median plane of the future animal.

Since in the egg of *Nereis* the sperm may enter at any point and since the first cleavage plane passes through this point, the struc-

ture of the ovocyte of *Nereis at the time of insemination* must be the same in all meridians. This, I believe, has an important bearing on theories of germinal areas in the cytoplasm, of prelocalization, and of precocious segregation. The determination of bilaterality follows fertilization.

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PALMEN'S ORGAN AND ITS FUNCTION IN NYMPHS OF THE EPHEMERIDÆ, HEPTAGENIA INTERPUNCTATA (SAY) AND ECDYURUS MACULIPENNIS (WALSH).

J. E. WODSEDALEK.

Introductory Remarks.

Our knowledge concerning the tracheal system in the Ephemeridæ dates back to the time of Swammerdamm (1752), but the existence of this interesting modification, Palmen's organ, found only in the tracheal system of this group of insects, was not known until comparatively recent times. Swammerdamm in his "Bibel der Natur" gives a large figure (Plate XIV.), showing in some detail the internal anatomy of a may-fly nymph, but the Palmen's organ and even the four tracheal tubes directly leading to it, if present in that species, apparently escaped his observation. This omission was no doubt due to an imperfect dissection; for, upon closely observing his representation of the air tubes in the head of the nymph he figures, one can detect a single projection leading from the main tracheal tube on the left, which corresponds somewhat to one of the four tubes normally leading to this organ; the other three tubes and the organ itself were doubtless destroyed in his preparation, and hence not represented in his figure.

The presence of this chitinous structure was first noted by Dr. J. A. Palmen (1877), after whom the organ is named, and in his work he says: "Die vier im Scheitel zusammenstossenden Aeste bilden in ihrem Kreuzpunkt einen rundlichen, aus concentrischen Chitinschichten bestehenden Körper, dessen Bedeutung ich nicht kenne." On Plate I. (Fig. 7) he gives a figure of the head and thorax of the nymph of *Cloëon dipterum* L., showing the location of this organ in its relation to the four tracheal tubes of the head, without making any attempt to describe it. He makes the statement that the tracheal system is essentially the

same in the twenty-three species which he examined. It is not entirely safe, however, to infer from this that the prominence of Palmen's organ is essentially similar in these various species.

The species upon which the present study is based are *Heptagenia interpunctata* and *Ecdyurus maculipennis*. These two forms are very closely allied, not only in matters concerning this organ, but also in their natural habits and general behavior, and the present paper will concern itself with nymphs of *H. interpunctata*, unless otherwise specified.

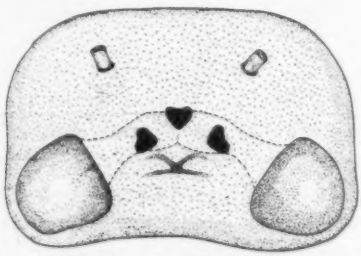

FIG. A. Head of *H. interpunctata* nymph. Basal joint of antennæ only drawn. The brain is drawn, dotted, under the three ocelli just posterior to which is shown the Palmen's organ and the four tracheal tubes leading into it.

Fig. A shows the relative position of the organs in the head of a nymph. Palmen's organ together with the tracheal tubes leading to it can be readily seen through the chitinous covering, especially in the newly moulted specimens, by placing them under a binocular. It is symmetrically located between the two large compound eyes and a little posterior to the brain. Fig. 1 shows the organ in its relation to the entire tracheal system of the head. It has been the fortune of the writer to be able to make a perfect dissection of the system, the first time merely through an accident. Upon placing a specimen which had been dead for some time under a binocular almost the entire tracheal system of the insect became visible through the transparent chitinous covering. The muscles and all the other soft tissues had sufficiently decomposed to form a sort of liquid mass. The thin hypodermal walls surrounding the air tubes too had disintegrated, and practically all that remained in good condition was the exoskeleton and its internal continuation, the tracheal system. The location

and arrangement of the more important parts of the system were carefully noted and a diagram indicating the relative position of the main tubes was sketched. The external covering was carefully broken between the pro- and mesothorax and a gentle pull on the anterior edge of the head removed it, fully exposing the air tubes which remained in position. The macerated mass was carefully washed off and the tracheal system being completely filled with air presented the most beautiful silvery effect against a dark back-ground. Even the very finest branches remained, but no attempt was made to include them in the figure.

Description of Palmen's Organ.

Gross (1903) attempts to describe the organ in considerable detail; this description and his ideas in general are not fully corroborated by the results of my studies. He says in part: "Reconstruiren wir jetzt aus den besprochenen Schnittbildern das ganze Organ, so erhalten wir folgendes Gesammtbild. Ein Körper, der im Längsschnitt kurz elliptisch, im Quersschnitt ungerfähr kreisförmig ist, setzt sich aus 14 concentrischen, aus zartem Chitin bestehenden Schalen Zusammen, die an ihrer Innenfläche mit feinen Härchen dicht besetzt sind. Das Ellipsoid ist aber kein vollkommen geschlossenes. Vielmehr ist es von vier Seiten her [sehr] tief ausgehöhlt. Das ganze ist in den Kreuzungspunkt von 4 im Scheitel des Hinterkopfs zusammentreffenden Tracheenästen eingeschaltet, und zwar so, dass die Luft zwischen den Schalen frei circuliren kann, wenn auch durch die grosse Zahl der Härchen einigermaassen behindert. Ganz ähnlich gestaltet wie bei *Ephemera vulgata* L. fand ich das Palmen'sche Organ noch bei *Baëtis rhodani* Pict., *Heptagenia sulphurea* Müll., ferner bei den Larven einer *Caënis* sp. und einer *Chirotonetes* sp. Einige geringe Abweichungen in der Gestalt bei *Baëtis rhodani* konnte ich nicht hinreichend genau feststellen, un sie hier zu besprechen, da ich mir nicht genügend Material beschaffen konnte."

The Palmen's organ in both *H. interpunctata* and *E. maculipennis*, is not composed of concentric shells nor are there any hairs present on the inner surface of the scales which Gross describes and pictures in allied species. Well prepared slides

of cross sections show that the organ is not perforated with air passages but is a continuous mass of chitin in which the differentiations are due mainly to variation in density of color. Sections of adult specimens were also made and carefully examined, but no difference in the structure of the organ could be detected. Dr. Gross has no doubt mistaken the clearer areas or concentric layers for air passages and the darker layers for separate solid areas forming the scales from which the hairs protrude.

Fig. 4 shows the external dorsal appearance of the organ and its relation to the four tracheal tubes, the entire structure being enveloped by the hypodermal layer; Fig. 5 is a horizontal section of the same. The description of the organ can be best understood by studying it in connection with its development and growth. It is a well understood fact that the tracheal system in insects is formed by the invagination of the ectodermal layer. As to the origin of Palmen's organ I am not at all certain for embryonic material has thus far in this study not been available. The appearance of the structure of the central portion of the organ suggests that, during the process of the development of the tracheal system, the four large tubes leading to the organ (Fig. 1) come together at a common point; here the blunt ends of the invaginated portions, the tracheæ, surrounded by the hypodermis, fuse and secrete this common center. From the various cross sections of which Fig. 8 is typical, it can be inferred that the two posterior tubes come together first and that a portion of the center is secreted before it is met by the two anterior tubes.

In the many sections of *H. interpunctata* and *E. maculipennis*, which were examined, the center of the organ does not show any ring-like structure, but is an irregular mass which is apparently secreted before the larva casts the first lining of its tracheal system. At the time of this first ecdysis which is accompanied by the shedding of the inner lining of the air tubes, this central mass is larger than the openings in any of the four tubes and hence the impossibility of its being cast out of the body. Shortly after the casting of the inner lining of the tracheæ, the hypodermal cells surrounding the tubes undoubtedly begin to secrete the new chitinous wall. The hypodermal layer surrounding the central mass, the beginning of the Palmen's organ, is continuous

with the layer surrounding the air tubes and apparently begins active secretion at about the same time. The different conspicuous rings which are shown (Figs. 5–9) are sections through the concentric layers of the organ and are directly correlated with the various moults. Further evidence of this correlation is obvious from the fact that the number of rings is directly in proportion to the size of the insects themselves. An examination of the sections figured shows that the hypodermal cells surrounding the organ are much larger than those enclosing the tracheæ, and hence, the greater the secretion of these larger cells; from this results the greater thickness of the chitinous layers of the organ as compared with that of their continuations, the walls of the tracheal tubes. Coincident with this increase of volume of the organ, the cells surrounding it must necessarily multiply as they are pushed outward. Thus, by means of successive periodic secretions the Palmen's organ is built up; the old layers of the organ are not cast off as are the walls of the tracheal system, with which they are continuous.

Gross in commenting on the function of Palmen's organ says: "Ich glaube deshalb, dass für das räthselhafte Organ keine Erklärung gefunden werden kann ohne Berücksichtigung des Nerven. Nehmen wir aber an, dass dieser wirklich zu dem Organ gehört, so kann dieses nichts anderes sein als ein Sinnesorgan. Da es aber, wenn auch ziemlich direct unter der Hypodermis—von dieser nur durch wenig Fettkörper getrennt—doch jeden Falls im Innern des Körpers der Thiere gelegen ist, kann es von allen uns von andern Thiergruppen bekannten Sinnesfunctionen nur denen eines Gleichgewichtssinnes dienen." Up to the present study no experimental work on the organ has been attempted with the view of obtaining evidence as regards its function. Gross also says: "Man könnte meinen, der Beweis für die Richtigkeit der von mir versuchten Deutung des Organs liesse sich vielleicht durch zweckmässig angestellte Versuche erbringen. Das erscheint mir aber ziemlich aussichtslos. Es wäre ja gewiss nicht unmöglich, das recht oberflächlich gelegene Organ zu zerstören, nachdem man vorher seine Lage so genau festgestellt hat, dass man sie schon von aussen am lebenden Thier angeben kann. Aber ich fürchte, dieses Experiment wird nicht viel helfen. Stellt

sich nach dem operativen Eingriff irgend eine Aenderung der Flugweise ein, so kann diese auch durch die Verletzung an und für sich bewirkt sein. Wir wissen aus der experimentellen Gehirnphysiologie der Vertebraten zur Genüge, in welche schwere Irrthümer man geraten kann, wenn man die Verletzung oder Zerstörung eines Organs oder Organtheils als reinen Versuch betrachtet. Während man aber bei einem Wirbelthier wohl warten kann, bis die störenden Nebeneffecte des operativen Eingriffs verschwunden sind, so scheint mir das bein einer 'Eintagsfliege' kaum möglich zu sein. Selbst ein nicht zur Begaltung gelangtes Exemplar dürfte in der Gefangenschaft nur zu bald eingehen. Auch würden die Thiere wohl kaum den Hochzeitsflug aufnehmen, wenn man sie nicht in die ihnen zusagende, natürliche Umgebung und unter Artgenossen bringt. Thut man dies aber, so würden einem die Versuchsthiere gar zu leicht entschlüpfen, nachdem sie einmal aufgestiegen sind. Ebenso wenig Erfolg verspreche ich mir von dem Versuch, die Function des Organs durch Verkleben der in die Kopftracheen führenden Stigmen festzustellen."

Removal of the Organ.

Experimental work on the removal of the organ did, as Gross said, at first seem impossible. It is needless to say that the task was very tedious and at the outset far from encouraging, this was mainly due to the small size of the organ and its close proximity to the brain. At first the cauterizing method was used but without satisfactory results, then two very fine platinum needles which were attached to the two wires leading from a galvanic battery were employed. The apparatus was provided with a resistance box so that the voltage could be varied at will. In this method the end of one needle was turned into a small loop through which the sharp point of the other was inserted, thereby completing the current, heating the sharp point intended for the operation, and at the same time, greatly facilitating the necessary steady manipulation of the outfit. The hot point of the needle would be brought directly over the organ and then a rapid insertion and withdrawal of the point of contact followed. It was impossible at each attempt to destroy the organ owing

to its natural instability. A few specimens from which the organ had been thus entirely removed, lived a sufficient length of time to enable studies of the behavior of the individuals, and of the regeneration of some of the destroyed parts.

Becoming more thoroughly familiar with the structure and exact position of the organ in its relation to the vital parts of the head, a more simple method was devised. By means of two very fine and sharp-pointed needles a small slit can be made through the chitin above the organ and then, inserting a needle at each side between the posterior and anterior tracheal tubes leading to the organ, it can with some practice, be entirely removed; this treatment apparently causes the insects but little pain. The four tracheal tubes were usually separated near the organ though sometimes they would break off near their juncture with the main longitudinal tracheæ. In special cases, for studies of regeneration of the organ, the four tubes were broken off at their immediate attachment to the organ or at various definite distances from it. This was possible by pressing the two points of the needles on either side of the place where the break was desired. Bleeding was very rare and usually the edges of the chitinous slit were brought so close together that the detection of the wound was rendered almost impossible.

After treatment by this method the activity of the nymphs when placed back into the water did not seem to be impaired by the operation, and the wounds healed over within a few days. By this method not only was the removal of the organ assured, but mortality was reduced to a minimum. In one set of experiments forty-nine out of fifty specimens operated on lived for more than two months after the operation. It might be said in this connection that no regeneration of the organ takes place. The ends of the broken tubes heal over within two or three weeks and with the exception of a few small air tubes which grow out from the blunt ends of the four tubes, during the same time, no further growth was observed in any of the specimens as long as four months after the organs had been removed. Fig. 3 is drawn from a nymph in which the tracheæ were broken off at their point of contact with Palmen's organ, they almost touched but no regeneration of the organ

took place, nor was there a union formed between the different tracheæ Fig. 2 is of a specimen in which the tracheæ were broken at quite a distance from the organ; again, no growth beyond the covering over of the broken ends and the formation of a few small tubules took place.

Comparison of the Behavior of Normal and Operated Specimens in Relation to the Function of Palmen's Organ.

In my previous papers (Wodsedalek, '11 and '12), the behavior of *H. interpunctata* nymphs has been discussed in considerable detail, and hence only the more important phases of the behavior of this insect which directly concern this problem will be cited here. The nymphs are decidedly negative in their phototactic response in all gradations of light, varying from ordinary daylight to very intense electric illumination. Their thigmotactic propensity, or tendency to come in contact with and cling to objects, is especially pronounced. In their natural environment the nymphs are never seen swimming freely about in the water, even when observed in their favorite places in which they occur in great abundance. In their natural habitat they are always found clinging to the under surfaces of small rocks, and this same position is regularly assumed by all normal ones in the aquaria of the laboratory. When a stone, to which the specimens are attached is inverted in the water, the insects soon make for its under side, many of them doing this as the stone is being turned over. This is also true of normal specimens in the dark-room, and hence it is obvious that this tendency of the nymphs to cling to the lower surfaces of rocks, with their dorsal side downward, is not due entirely to their negative reaction to light. It is unquestionably due, in part, to a definite power of orientation independent of phototaxis.

Specimens from which the Palmen's organ was removed react to light in practically the same way as do the normal specimens. Their thigmotactic inclinations, too, do not seem to be impaired. However, when the insects are taken into a very shaded or a dark-room the difference in orientation becomes quite obvious. When a stone to which the insects are attached is

inverted in the water, or when the specimens are dropped on a stone in the water in a dark-room they remain on the upper surface or on the sides of the rock for a considerably longer time than do the normal individuals By the removal of the organ the nymphs have no doubt lost, to some extent, their usual keen sense of orientation, for under such conditions they would be seen on the top, sides or any part of the rock for hours, days, and even weeks after the operation had been performed. The same was true of every lot experimented with. It was also noticed, with several lots of operated specimens, that the tendency to remain on almost any part of the stone was gradually diminished and that after several weeks and in some cases about two months there were comparatively few individuals to be seen on the upper surface, regardless of the fact that in some special experiments the stone would be inverted at every observation with the view of bringing more specimens to the upper surface with little disturbance. This growing partiality to the lower surface of the stone does not lessen the significance of their former behavior, for, from my studies on the power of the formation of associations in the nymphs of *H. interpunctata* (Wodsedalek, '12) it was found that they gradually formed several types of associations. The associations formed in these experiments were in connection with their thigmotactic inclinations, which were in great part responsible for the gradual decrease of the number seen on top, and the gradual diminishing of the time the various individuals required to retreat to the lower surface.

In another paper (Wodsedalek, '12) on the natural history and general behavior of these insects I have discussed their thigmotaxis in considerable detail. It was learned from a simple experiment that their thigmotactic propensities are best satisfied when their dorsal as well as their ventral surfaces are in contact with some object. "When several specimens are placed in an aquarium they mass together into clusters where they remain for hours, and if recently collected, even days. As soon as a rock or any other object is placed in the water the loose forms swim toward it, while considerable time often elapses before the masses are broken up. Two long bricks were placed one over the other in a basin of water and between them were placed small pebbles

varying in size so that the space gradually varied in thickness from one end to the other. Then a large number of specimens were put in the water and after a short time it was found that nearly all of the specimens were attached to the lower surface of the upper brick with their dorsal sides downward, and a large majority of the specimens were in that part of the wedge-shaped space where their backs came in contact with the brick below." The operated specimens in their wandering about over the surface of the stone accidently came into such a place where their backs came in contact with the floor of the basin. This stimulus naturally appealed to their thigmotactic propensity and hence the greater tendency to remain on that portion of the rock. It seems only natural, therefore, that an association would be formed between this more satisfactory environment and the lower surface of the stone. It is not altogether improbable however, that such a habit had already been partially formed before the operation took place.

Further evidence for the fact that this thigmotaxis is largely responsible for the gradual disappearance of the insects from the upper surface, is apparent from the results obtained in some checking experiments. In those experiments the stone was suspended in the water so that the backs of the nymphs could not come in contact with other objects. The results were surprising and all remaining doubts as to the function of the Palmen's organ in the nymphs were resolved. As long as the experiment was continued the specimens remained quite evenly scattered over the entire surface of the suspended stone. A similar experiment was tried with the normal specimens, also in the dark chamber, and practically all of the specimens remained exclusively on the lower surface. It is only natural, then, to conclude that Palmen's organ has a great deal to do with the orientation of these insects. That this unusual behavior is not due to the shock the insects receive from the operation was proven by the fact that when other parts of the head and body were destroyed no comparable results in behavior took place.

Although the foregoing results are thoroughly convincing as to the function of the organ in these nymphs, further results of observations on behavior relative to the rôle of the organ may

be cited. When the specimens are collected and dropped into a dish of water many of the individuals fall to the bottom with their ventral sides upward. This toppling over is even more obvious when the specimens are placed in a dish of water near a light. In their attempts to get away from the light and repeatedly clawing at the opposite end of the dish the specimens become exhausted and very frequently when the clawing movements cease the apparently lifeless individuals fall to the bottom, dorsal side downward. This period of rest corresponds somewhat to the death-feigning instinct of the insect. By vigorously stirring up the specimens or throwing them into water having a temperature to which the specimens are not accustomed, or into relatively strong chemical solutions of various sorts, as acids, salts, alcohol, etc., practically all of the specimens fall into this momentary, rather stiff, inactive state and slowly descend to the bottom of the dish. In so doing almost all of the specimens topple over and fall down head-first, ventral side up and on the average, at an angle of about 45 degrees. It might also be mentioned here that nymphs which are found dead in the aquaria lie almost invariably with their ventral side up. On the other hand, the turning over is under similar conditions far less frequent among the specimens from which the organ had been removed. If two groups of freshly killed specimens are taken, all of which have been cleaned and their appendages arranged, the one group normal in every way, the other having the Palmen's organ removed, we find by allowing them to descend through a deep jar of water that almost invariably the former topple over and settle on the bottom ventral side up, while the latter equally as frequently reach the bottom and remain there with their ventral side downward.

Concluding Remarks.

The results of the foregoing experiments show conclusively that the organ, as small as it is, plays a very important rôle in the behavior of the nymphs upon which these experiments were performed. This is doubtless due to the weight of the chitinous mass whose pressure seems, to a large extent, to control certain orientation of the insects. Gross (1903) gives a figure of the

cross section of the head of a may-fly showing the position of Palmen's organ in relation to the other parts, and in his discussion says,—"Unter dem Palmén'schen Organ verläuft nämlich bei allen 5 von mir untersuchten Ephemeridenspecies ein starker, vom Gehirn kommender Nervenstrang. Seine Lagebeziehungen ergeben sich aus Fig. B, die einen Medianschnitt durch den Kopf einer Ephemera vulgata bei schwacher Vergrösserung darstellt. Der erwahnte Nerv (*np*) verlauft in der Medianlinie vom Gehirn (*g*) nach hinten unter dem Palmén'schen Organ (*p*) hindurch und heftet sich hinter ihm an der Körperwand an. In einem Theil seines Verläufs liegt er direct auf dem Nervus recurrens (*nr*) des unpaaren sympathischen Nervensystems."

Careful examination of many nymphs showed no evidence of the presence of the two large nerves which Gross speaks of as present in the imaginal species which he examined; this was also true of the adult specimens which I examined. It appears from his discussion of the subject and from his figure (page 98), that what he speaks of as nerves may possibly be the two muscles which play an important part in the movement of the head. The posterior attachment of these muscles to the exoskeleton evidently corresponds to the attachment of the large nerves he misrepresents. In my preparations very thin sections were made, but no signs of nerves extending directly from the brain to the organ were detected. Taking the structure and function of the organ into consideration we should not expect the presence of such nerves. A mass of rather loose tissue exists between the organ and the brain, and the two are loosely united by means of connective tissue. It is the writer's opinion that the chitinous organ being so loosely supported by the four tracheal tubes exerts a pressure on the surrounding tissues, whereby the disturbing stimulus reaches the central nervous system. The observations mentioned on the descent of nymphs in various conditions, through the water, particularly the death-feigning and the dead individuals, seems to indicate that the orientation is also, in part, a self-directing process, that is, by the presence of the organ the nymph is swerved into position—a matter of physical equilibrium.

Gross' theory that the organ functions only in the adult speci-

mens seems quite untenable. Aside from the results of my experimental work arises another question. Why should this structure occur and persist in very small nymphs, and grow in relative proportion during the comparatively long nymphal stage of two, and in some cases three years, for the purpose of becoming functional only after the nymph metamorphoses into its short-lived adult stage, when all the other modifications which are of a direct advantage to the adults develop during the comparatively short time immediately preceding the transformation?

The extent of the functions of this organ in the adults thus far remains unknown. Miall (1895) in speaking of the Ephemeridæ gives the following quotation: "The recently emerged fly," says De Geer, "settles on trees, plants, walls, etc., near the water which harbored the larva. Here it fixes itself by the hooks of the feet, usually with the head downwards, and rests until the last or sub-imaginal moult is at hand." My observations of the behavior of adult may-flies are to some extent in accord with those of De Geer, however, no theory as to the probable function of the organ in the adults can be propounded, unless it can be supported by reliable results of experimental work. A large number of nearly full grown nymphs from which the Palmen's organ had been removed are now in the aquaria with the view of making a study of their behavior, when they emerge as adults in comparison with that of the normal individuals.

Among the twenty-three species in which Palmen (1877) noted the presence of this organ, there are several free swimming forms, and at this time, it is difficult to say just what part Palmen's organ plays in those forms during their life history as very little is known of their natural habits.

I am greatly indebted to Professor William S. Marshall for suggesting this problem, and also, for his help and earnest interest in the progress of the work.

ZOÖLOGICAL LABORATORY,
UNIVERSITY OF WISCONSIN.

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EXPLANATION OF FIGURES.

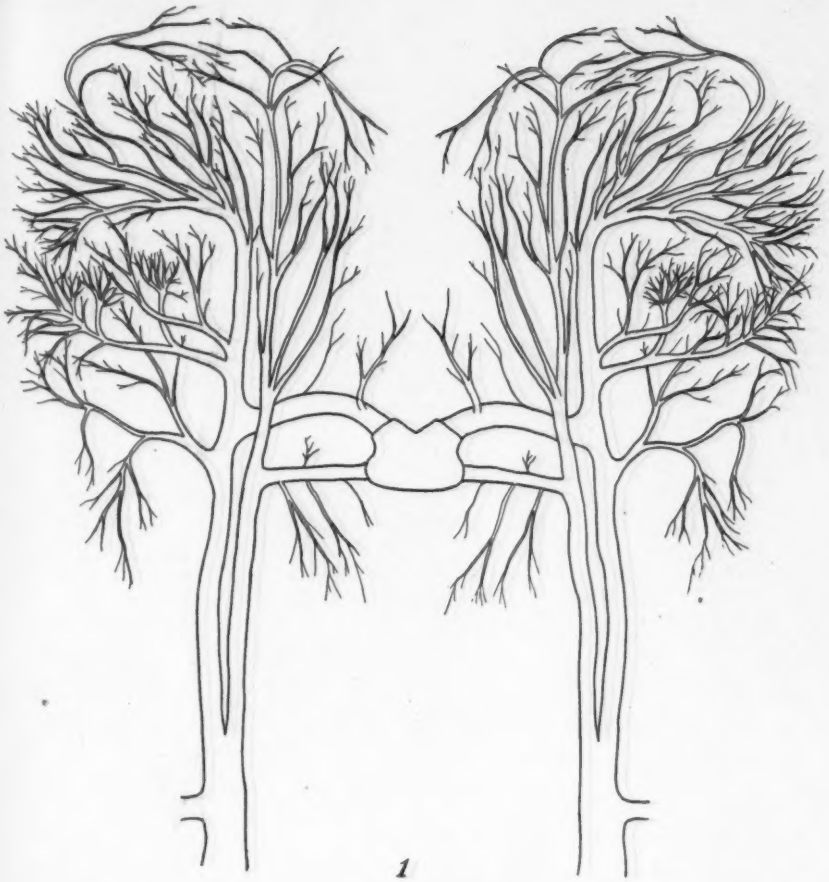
PLATE I.

All drawings (except Figs. 2 and 3) made with a camera-lucida. ×240.

FIG. 1. Palmen's organ in its relation to the tracheal system in the head of the nymph *H. interpunctata*. × 60.

FIG. 2. Sketch drawn from a specimen which had the organ removed and the four tracheæ broken off near their juncture with the main longitudinal tubes.

FIG. 3. Sketch drawn from a nymph in which the tracheæ were severed at their point of contact with the organ.



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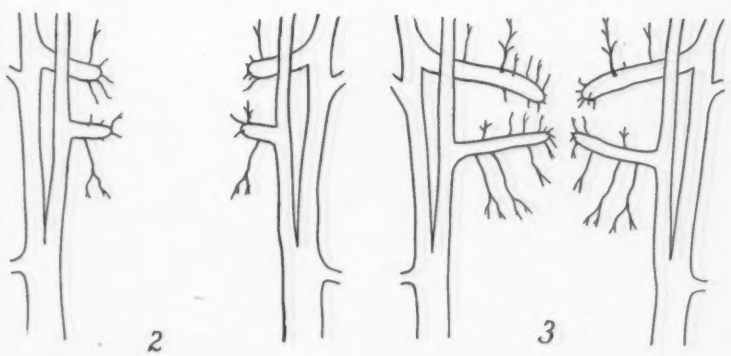

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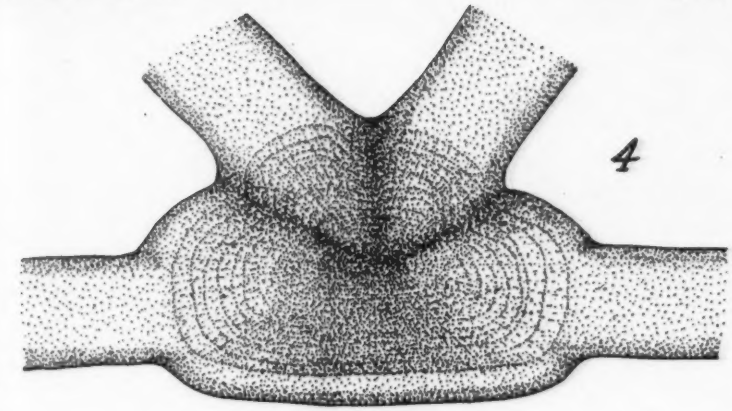
PLATE II.

FIG. 4. Representation of the entire organ surrounded by hypodermis, as it appears in a mounted specimen. Circular bands can be seen, especially at the edges of the organ, owing to the fact that we look at the vertical portion of each deeply colored part. In this view the large light areas appear at the entrances of the tracheal tubes; this is due to the fact that we look through a comparatively thin portion of the chitin in those regions owing to the direct extension of the cavities of the tubes into the organ. The darker areas appear as such because of their thickness; each is a concentric mass around the organ and forms the partition between the cavities.

FIG. 5. A horizontal section almost directly through the center of the two posterior tubes and a little above the center of the two anterior ones. It is only natural, therefore, that the two posterior tracheæ should lead to the solid central mass. The entrances of the two anterior ones are not in the same plane with that of the posterior pair and therefore the innermost portion of their cavities are not represented in this section. The gradually increasing diameter of each cavity is understood when we recall the development of the organ and the tubes leading to it.

FIG. 6. A horizontal section through the ventral projection of the organ which is apparent in Figs. 7 and 8. The central part of this figure appears clear because the section was quite thin and the cut parallel with the light portion of one of the concentric layers.



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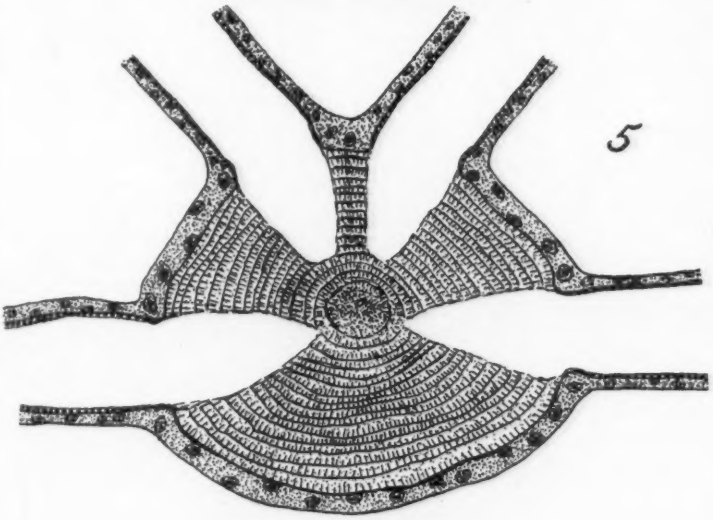

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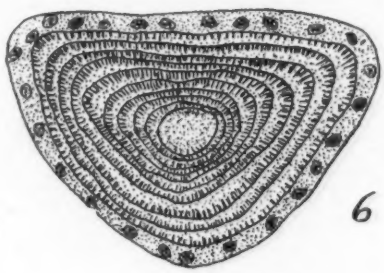

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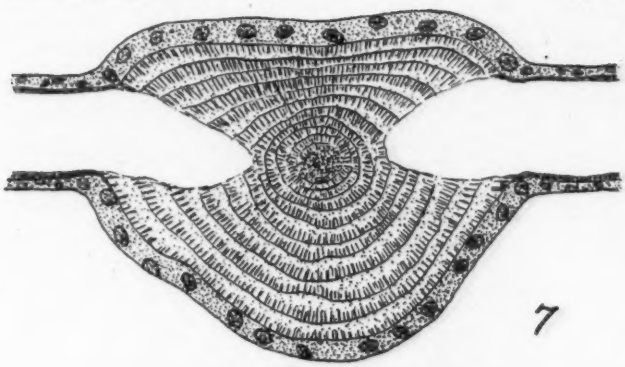
PLATE III.

FIG. 7. A transverse section cut near the center of the posterior pair of cavities.

FIG. 8. An oblique transverse section cut through the front part of the posterior tube cavities and through the tips of the anterior cavities.

FIG. 9. A still more anterior view, only the cross sections of the two deeper portions of the anterior cavities being in evidence.



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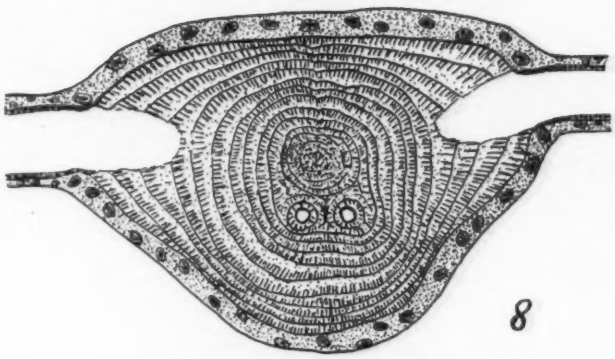

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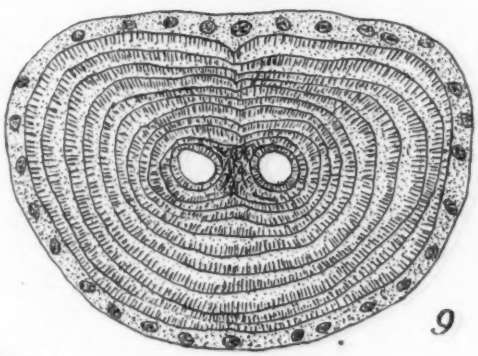

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